Evaluation of the Effect of Bacteriophages on Mosquito Larval Development

By
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ABSTRACT

Mosquitoes are medically important arthropod vectors of arboviruses and *Plasmodium* parasites with half of the human population globally at risk of mosquito borne diseases. Due to the lack of efficient vaccines and the growing problem of insecticide resistance, alternative approaches to curb mosquito transmitted diseases are needed. An area of increased interest is the mosquito microbiome. Mosquitoes rely on microbes for larval development and the microbiome has also been shown to affect vector competence. In order to better understand the functions and effects of the microbiome, new tools must be developed to better target and investigate specific species of the mosquito microbiota. In this study, the potential use of bacteriophages as a tool to modulate the microbiota composition in *Aedes aegypti* larval breeding water was investigated. Mosquito larvae were placed in gnotobiotic conditions where different bacteria species were inoculated into the breeding water to study the effect that bacteriophage-mediated modulation of the microbiota had on time to pupation. Six bacteria species were used: *E. coli* W3110*, Enterobacter spp., Serratia spp.,* two *Microbacterium* species, and *Arthrobacter spp.*. Larval development was first observed when only one of the six bacteria species was added to the breeding water and it was shown that in the *E. coli* W3110 and *Enterobacter spp.* treatments, larvae reached pupation the fastest with the highest survival rate of the six species. The two *Microbacterium* species mediated the slowest pupation rates and the lowest survival rates. The *E. coli*-infecting bacteriophage, T7, was then added to the breeding water in a separate *E. coli* treatment as a bacteria-bacteriophage proof of concept. Delays in larval pupation and lower survival rates were then observed in the bacteriophage treatment.

Bacteriophages targeting *Enterobacter spp.* and *Serratia spp.* were then isolated and the *Enterobacter spp.* were sequenced. Sequencing revealed that the two isolated bacteriophages were induced from two prophage regions of the *Enterobacter spp.* genome.

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INTRODUCTION

Mosquito Burden

Vector-borne diseases account for about 17% of the global burden of all infectious diseases. Attempts to treat and prevent infection is a heavily researched area with various levels of success. One of the most successful interventions has been to control the vectors, mostly through various pesticide uses. However, due to the long use of these pesticides, an increasing number of mosquito populations have been gaining resistance to these pesticides and new interventions to control mosquito populations must be sought (Liu 2016).

Malaria

The *Anopheles* mosquito is the primary mosquito vector responsible for the transmission of malaria in most of sub-Saharan Africa. Of the 400 different species, about 30 are malaria vectors of high importance (WHO 2019). Of those 30, *A. darlingi* (South America)*, A. gambiae s.s., A. funestus* (sub-Saharan Africa)*,* and *A. culicifacies* (South Asia) are considered the dominant malaria vectors in their geographic area (Kiszewski et al. 2004).

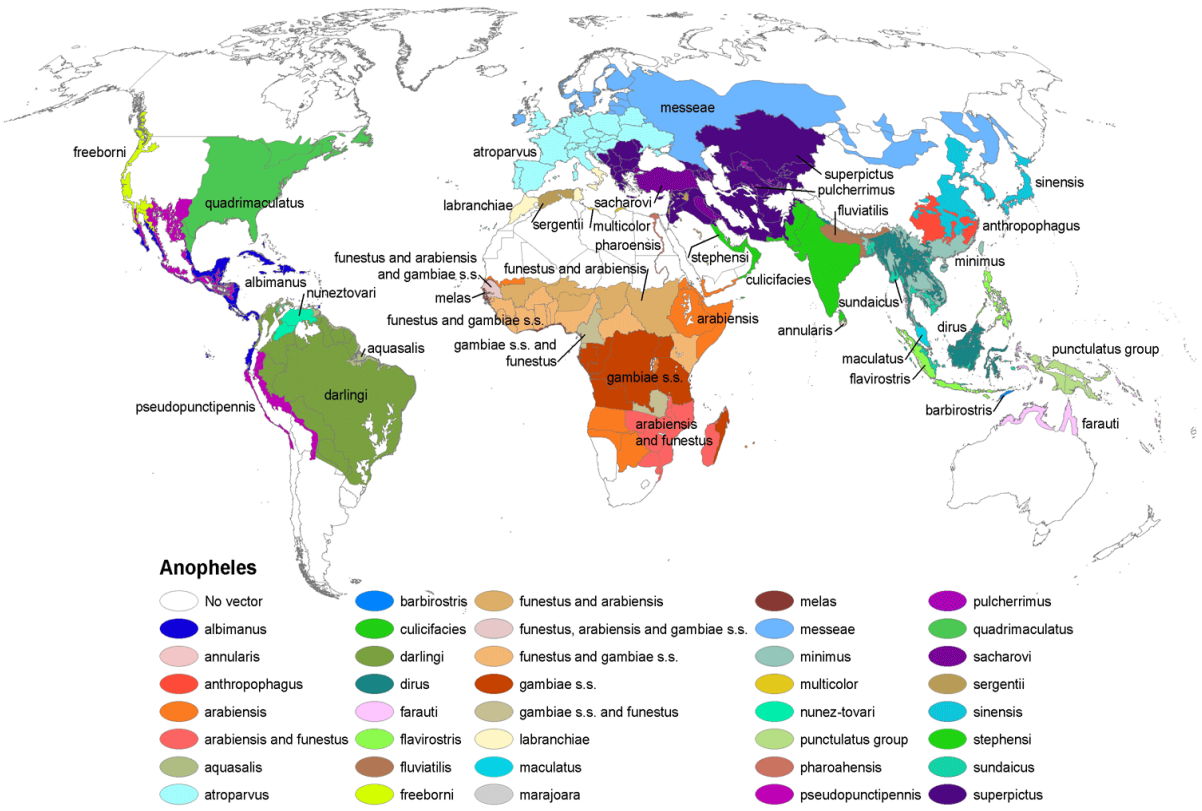

Figure 1: Global distribution of dominant or potentially important malaria-transmitting *Anopheles* species (Kiszewski et al. 2004)

Though most *Anopheles* do not have a preference between blood-feeding on humans or other animals, *A. gambiae* and *A. funestus* are two species that do show a strong preference for humans, thus are two of the most efficient malaria vectors (WHO 2018). As seen in Figure 1, *Anophelse* species that can transmit malaria are found worldwide, and even if a region had eliminated malaria or if it is not endemic to the area, there is still a risk of introduction or re-introduction.

Malaria is caused by the *Plasmodium* protozoan parasite, of which there are five species that cause malaria in humans. *P. falciparum* and *P. vivax* are two of those species that are the most cause for concern. In 2017, *P. falciparum* accounted for 99.7% of

malaria cases in the African region. *P. vivax* accounted for 74.1% of malaria cases in the Americas (WHO 2019).

Plasmodium has two lifecycles, one in the mosquito, the other in the human host. There are some differences in the lifecycle depending on the *Plasmodium* species, but all still follow a similar pattern. When a human host is bitten by a mosquito that is infected with the parasite, the sporozoite stage enters the human bloodstream from the mosquito salivary glands and will travel to the liver to infect the hepatocytes cells. Once in the liver, the sporozoites develop asexually into exoerythrocytic merozoites over the course of 5-15 days. *P. vivax, P. ovale* and *P. cynomolgi* have a dormant stage where hypnozoites do not mature and may stay in the liver for months or even years before the parasite develops into exoerythrocytic schizogony like other *Plasmodium* species that then release merozoites into the bloodstream. Merozoites infect red blood cells and develop into trophozoites in the erythrocytic cycle. The trophozoites develop into schizonts, which rupture to release more merozoites. The merozoites can also enter a sexual differentiation stage and develop into gametocytes. It is the gametocytes that are taken up by a mosquito in a bloodmeal.

Once in the mosquito, the parasitic gametocytes move through the mosquito digestive tract with the blood meal and sexually reproduce in the mosquito gut within minutes of taking the bloodmeal. The resulting zygotes develop into ookinetes that are mobile and move to penetrate the peritrophic matrix 24-36 hours later. After penetrating the matrix, the ookinete continue to move through the midgut epithelium. Once the ookinetes reach the basal side of the epithelium, they develop into oocysts that develop under the basal lamina of the midgut to develop and mature for about 10-12 days. The

oocysts then release sporozoites numbering in the thousands that travel throughout the mosquito's body through the hemolymph, infecting cells throughout the mosquito body. It is only when the sporozoites travel to and infect the mosquito's salivary glands that the entire infectious lifecycle is able to start over again. (CDC 2018, Mikolajczak et al. 2015, Wahlgren & Perlmann 1999, Luckhart et al. 1998).

The first symptoms of malaria are nonspecific and include fever, headache, chills, dizziness, and abdominal pain. Severe malaria symptoms are more important to take note of, but are similarly nonspecific and include confusion, coma, severe anemia, or respiratory difficulties (CDC 2018). Cases can only be confirmed with a laboratory test. Laboratory tests can be done with microscope diagnosis, the gold standard, where the patient's blood is spread in a blood smear and parasites can be visually identified. Antigen detection is another test, also known as the Rapid Detection Test as they provide results in a matter of minutes and are much more readily available (CDC 2018).

Though recent interventions have been quite successful at halving the malaria burden in Africa since 2000, in 2017, 219 million cases of malaria were still reported. The number of malaria deaths stood at 435,000 (WHO 2019) and malaria still has an economic burden of $12 billion dollars annually worldwide (CDC 2018).

Dengue

Aedes aegypti is another mosquito of high public health importance. *A. aegypti* is the primary vector of many viral diseases such as yellow-fever, Chikungunya, and dengue fever (Aitken et al. 1979, Lamballerie et al. 2008, Weaver & Reisen 2010). The mosquito is found worldwide, though originally evolved in West Africa. Its ecological

success has been due to globalization, widespread colonization, increased human populations, and cross-oceanic travels (Weaver & Reisen 2010). It thrives in dense human populated areas with limited water and sanitation infrastructure (Honório et al. 2009). *A. aegypti*'s preference for dense populations along with various other behaviors make it a very efficient epidemic vector. Not only are *A. aegypti* able to breed in close proximity to human through the use of collected water in old tires, trash, cement cisterns, and open septic tanks, they are also able to rest indoors and bite during daylight hours. There are two peaks times of biting, one for a few hours after sunrise and another several hours before sunset (Gubler 1998). *A. aegypti* females will also feed on multiple people for one blood meal. They will stop feeding if only slightly disturbed and then return to feed on the same or different person. This allows them to even transmit diseases if they probe at a person even without taking a full blood meal (Platt et al. 1997). *A. aegypti* is restricted to temperate zones as their eggs do not undergo winter diapause and are vulnerable to frost (ECDC 2016).

Dengue fever is caused by a single stranded RNA virus belonging to the Flaviviridae family and is the most common arboviral and vector-borne disease in the world. In order to infect a host cell, a Dengue virion must first bind to cell-surface attachment molecules and receptors after which the virion is internalized into the host cell through endocytosis. The viral glycoproteins can then mediate a fusion of the viral and host cell membranes due to the low pH of the endosome. Once the viral and host cell membranes have fused, the viral RNA can be released into the host cell cytoplasm. The vRNA is then processed by viral and cellular proteases and the resulting non-structural proteins replicate the viral genome RNA. The virus assembly happens at the endoplasmic

reticulum membrane to form immature virus particles. As these virus particles are transported through the host cell, they pass through the trans-Golgi network where the acidic environment promotes virus maturation. The mature virus is then released from the cell (Bäck & Lundkvist 2013).

When a mosquito takes an infected blood meal, the first host cells the Dengue virion must infect in the mosquito are the midgut epithelial cells. From there, the virus particles are then released into the hemolymph where they continue to spread throughout the mosquito body and infect other host cells. The virus must then spread and infect the mosquito salivary glands in order to continue to transmit the disease when it takes its next blood meal (Xi et al. 2008). The extrinsic incubation time of the Dengue virus in the mosquito is about 7-14 days, though the highest levels of virus titer in the salivary glands happens at about 12-18 days. This is the period that a mosquito is able to spread the disease most effectively and quickly (Salazar et al. 2006).

Dengue has been recorded as far back as the Jin Dynasty (265 to 420 CE) where a published Chinese encyclopedia described symptoms of and remedies for dengue (Nobuchi 1979).There are four recognized serotypes of dengue, DEN-1, DEN-2, DEN-3, and DEN-4, all have been isolated from field-collected *A. aegypti* (Gratz 2004). Recovery from one serotype provides lifelong immunity against only that serotype. Cross-immunity to the other serotypes does occur after recovery but is only partial and temporary. Subsequent infections by other serotypes increases the risk of developing severe dengue infections (Deen et al. 2006).

The general form of the infection produces nonspecific flu like symptoms such as fever, frontal headache, nausea and vomiting, weakness, and rash (Waterman et al 1989).

Dengue hemorrhagic fever (DHF) is much more serious and is most common in children under the age of 15 years. During the beginning stage of DHF, the symptoms match dengue fever making it difficult to determine a clinical diagnosis. However, with DHF, there is an increase in vascular permeability which leads to plasma leakage. About 24-28 hours after the initial fever starts to decrease, patients may exhibit severe abdominal pain, persistent vomiting, rapid breathing and bleeding gums (WHO 2014). Without appropriate treatment and support, patients can go into hypovolemic shock and risk death (Gubler 1998). All four serotypes have been associated with DHF and variations between the serotypes only have influence on disease severity (Gubler 1998).

Dengue has an economic burden of $1.8 billion annually worldwide (Suaya et al 2009) and there are 50-100 million cases reported annually (Gibbons & Vaughn 2002). The global pandemic began after World War 2 when Southeast Asia saw huge increases in global transmission due to the Pacific theater (Halstead 1980, Gubler & Trent 1993) and later intensified in the 1980s (Halstead 1992, Pinheiro & Corber 1997). Dengue infection was rare outside of Southeast Asia previous to the 1940's, but by the 1980s DHF was on the rise worldwide regardless if the region had no endemic dengue, one serotype present, or multiple serotypes present (Guzman & Kouri 2003).

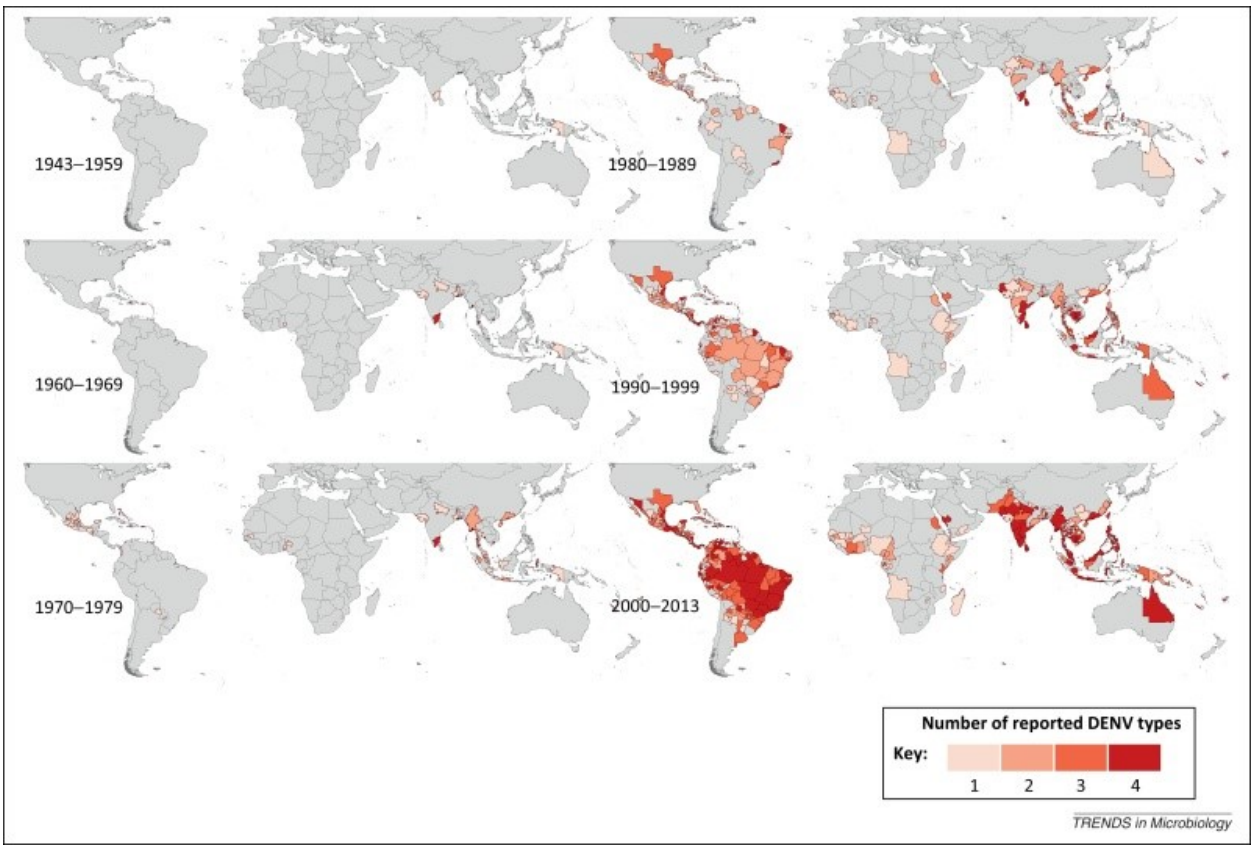

Figure 2: DENV co-circulation. As the key color becomes more saturated, the total number of reported DENV serotypes increases, a key indicator of hyperendemic transmission (Messina et al, 2014)

Prevention and Control

Malaria vaccine development started in the 1960's, but it has been proven difficult to develop a vaccine that confers long lasting immunity after one or a few exposures. The first report of a successful malaria vaccination happened in 1973, volunteers were immunized through the bites of about 1000 irradiated mosquitoes that had also been infected with *Plasmodium* sporozoites (Clyde et al. 1973). As interesting as this finding was, it became clear that vaccination through mosquito bites was not going to be a feasible disease control intervention.

Some of the reasons for difficulty of developing a malaria vaccine are the size and plasticity of the *Plasmodium* genome, the large variation of blood-stage antigens, and the need to ensure the vaccine does not set off a host immune response. The *Plasmodium* genome has about 23 million DNA base pairs, 14 chromosomes and has about 5,000 genes (Gardner et al. 2002). This genome is much larger than any of the bacteria or viruses that successful vaccines have been made for. Various genes are differentially expressed between the different life stages and their functions are not well understood. *Plasmodium* undergoes sexual reproduction and genetic recombination all of which lead to increased diversity and complexity of the *Plasmodium* parasites that make developing a single vaccine difficult. This is the same problem with blood-stage antigen diversity. In a single African village, there were over 200 recorded variants on one leading blood stage antigen (Takala et al. 2009). Without more careful molecular epidemiologic studies, simply choosing a few genetic variants to be used in a vaccine make it unlikely the vaccine would be cross-protective.

And like all other illnesses, the immune response to malaria contributes to the development of symptoms. Without careful diligence, a vaccine could increase the risk of a harmful inflammatory response especially with a vaccine targeting the blood stages. While there are several antimalarial drugs for therapeutic use, *Plasmodium* is notorious in developing drug resistance, thereby rendering the use of drugs for malaria elimination less effective.

Attempts to develop a dengue vaccine started since the viruses were first isolated in the 1940s (Sabin & Schlesinger 1945) and only recently has a dengue vaccine been approved for dissemination in the US (FDA 2019). This vaccine, Dengvaxia, however,

has only been approved for people who have already been infected with at least once. This is due to the vaccine was suspected of putting naïve populations at higher risk of severe infections (WHO 2017). There are also no therapeutic treatments for dengue. If an individual is infected and showing serious symptoms, the standard of care is only to keep monitoring the patient and providing supportive care. Some of the obstacles in developing a dengue vaccine for naïve populations have been the lack of an animal model and the necessity for a tetravalent vaccine. As mentioned previously, if a patient has preexisting antibodies to a different serotype than the one they are infected with, there is an increase in the risk of developing DHF (Sangkawibha et al. 1984, Burke et al. 1988). However, new studies and developments in vaccines have shown promise. At the Johns Hopkins Bloomberg School of Public Health, Dr. Anna Durbin led a clinical trial that demonstrated that a live attenuated vaccine elicited complete protection against DENV-2. Previous clinical trials showed the vaccine, TV003, elicited strong protection against DNV-1, 2, and 4. Since the findings of DENV-2 protection were released, Brazilian researchers began a Phase 3 clinical trial to investigate the vaccine's response and efficacy against naturally occurring dengue (Kirkpatrick et al. 2016).

With slow development in controlling vector-borne disease from the human side, vector population control has been one of the key interventions in reducing disease incidence. DDT was one of the first chemical vector-controls and was used extensively in the 1940s (Hemingway et al. 2002). The success of DDT's use and elimination of malaria in the USA led to the WHO's malaria eradication campaign in the 1960's. While DDT's use has been scaled back in the last few decades, new insecticides have been discovered and used. Pyrethroids are adulticides and were introduced for widespread use in the

1980s (Zaim & Guillet 2002). They are one of the most common insecticides used today. They are used for control methods such as bed nets and indoor residual house spraying and are popular due to low toxicity in humans and their effectiveness in killing insects (Hougard et al. 2002).

However, due to their prolific use, insecticide resistance is on the rise. Pyrethroid-resistant *A. gambiae* populations are prevalent in western and central Africa (Ranson et al. 2011).

It is thought there are two major mechanisms that are responsible for insecticide resistance; target site resistance and metabolic resistance. Target site resistance has been better researched and understood; pyrethroids delay the closing of sodium channel in insect neurons leading to paralysis and death (Ridl et al. 2008, Davies et al. 2008). Changes and mutations to that target site are what cause insecticide resistance. Metabolic resistance happens when one or more enzymes become more active and either sequester or detoxify an insecticide and thereby lowering its effectiveness. Metabolic resistance has not been as well studied, but some key enzymes have been identified in insecticide detoxification. Cytochrome P450 is a primary enzyme family identified in metabolic resistance (Feyereisen 2005). As more enzyme families that appear to play a role in metabolic resistance are discovered, there is the possibility of the discovering molecular markers for metabolic resistance. This would help in determining how metabolic resistance specifically impacts vector control interventions.

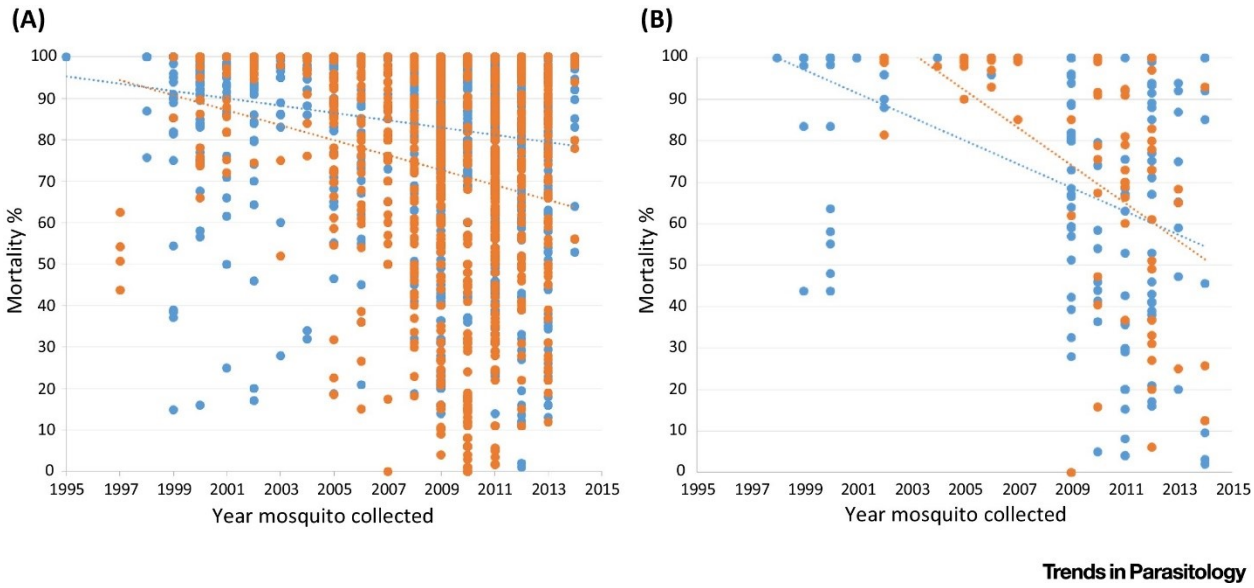

Figure 3: Changes in pyrethroid mortality in major African malaria vectors. Percentage mortality of (A) *Anopheles gambiae* sensu lato (s.l.) and (B) *Anopheles funestus* s.l mosquitoes exposed to 0.05% deltamethrin (blue) or 0.75% permethrin (orange) in World Health Organization (WHO) susceptibility bioassays (Ranson & Lissenden 2016)

Mosquito Lifecycle

Mosquitoes are part of the order Diptera, family Culicidae. Like all other Diptera, mosquitoes go through a complete metamorphosis and have four stages in their life cycle: egg, larvae, pupa, and adult.

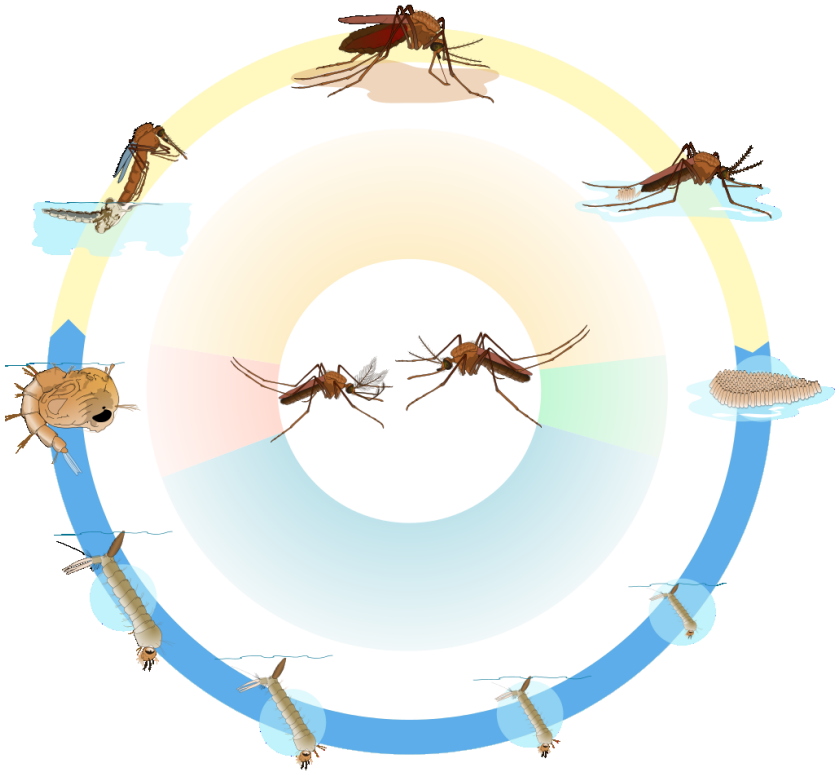

Figure 4: Mosquito lifecycle (Vector Disease Control International)

All mosquitoes require standing water to lay their eggs, though whether they lay in "floodwater" environments (temporary water habitats) or "permanent water" environments depends on the species. They lay their eggs directly on the water, some species lay their eggs in rafts, others lay their eggs individually. Eggs take a few days to hatch, though some can undergo diapause and overwinter as eggs. Once hatched, larvae will filter feed on microorganisms and organic material in the water; as they grow, they will go through 4 instars, or molts. Going through all instars can take about a week, though exact timing depends on the species and environment. After the fourth instar, the larvae will molt into a pupa. Known colloquially as "tumblers" due to their tumbling movement in the water, these pupae do not feed and will stay as a pupa for a few days as the mosquito metamorphosizes within. Adults will emerge from the pupa and fly off from

the water environment, many mating soon after. Both male and female mosquitoes will feed on plant nectars, but females are the only ones that will take a bloodmeal as it is necessary for egg development. Once the bloodmeal has been digested and the eggs have formed, the female mosquito will lay the eggs on a water surface, completing the lifecycle (Vector Disease Control International, CDC 2019).

Mosquito Microbiome

As with any animal, recent research has shown the importance of the mosquito microbiota for a variety of processes. The microbiota is necessary for larval development and pupation and can influence the vector competence of the adult mosquito.

Mosquito adults and larvae occupy two completely different environments with the larvae and pupae living in aquatic environments and adults occupying terrestrial environments, the composition of the gut microbiota between the two stages is also different. This is exacerbated by the fact that mosquito larvae flush out their microbiome as they pupate allowing a new community of microbes to colonize the adult mosquito's gut.

Mosquito larval acquire endogenous bacteria through various mediums. Most commonly is through the breeding water that they hatch into. As the larva filter feed in their environment, they also take in the bacteria present (Minard et al. 2013). Because the gut microbiome is so dependent on the surrounding environment, different studies tend to find different compositions in microbial communities. Yadav et al. (2015) surveyed wild mosquitoes in India and found that the most common bacteria found in the larval gut of *Aedes aegypti* and *Aedes albopictus* were the families Enterobacteriaceae, Bacillaceae,

and Pseudomonadaceae. Coon et al. (2014) performed studies with lab reared mosquitoes and found that in *A. aegypti* and *An. gambiae*, the most common bacteria found were Flavobacteriaceae and Microbacteriacea. Female mosquitoes can also pass down certain bacteria and fungi to their offspring through vertical transmission. This vertical transmission usually occurs through an infection of the egg cytoplasm (Sinkins 2004), colonization of the egg surface (Damiani et al. 2010), or through inoculating the breeding water (Lindh 2008).

Coon et al. (2014) also showed that bacteria are necessary in the larval stage for development. The bacteria in the gut gradually create a hypoxic environment, once the hypoxia reaches a certain level, it triggers a molting response in the larva and thus, progresses the development. However, despite the knowledge that a bacterial community is required for larval development, it is still unknown if each separate species of bacteria have different functions or effects on the mosquito.

Understanding the adult and larval mosquito microbiome has become a growing field of interest. Several studies have shown that changing the adult microbiota can affect vector competence. Dong et al. (2009) showed that a subset of immune genes were upregulated in septic adult *Anopheles gambiae* mosquitoes compared to aseptic mosquitoes. They also found that septic mosquitoes were less susceptible to *Plasmodium* infection as compared to the aseptic mosquitoes, thus suggesting that the anti-*Plasmodium* effect may be due to that upregulation of immune genes.

Mosquito Midgut Microbiome Composition

We are focusing on four different bacteria genera that have been consistently found in mosquito midgut microbiome. We looked at *Enterobacter spp.*, *Serratia spp,, Arthrobacter spp.,* and *Microbacterium spp.*.

	Aedes aegypti	*Aedes japonicus*	*Aedes triseriatus*	*Anopheles gambiae*	*Culex pipiens*	*Culex restuans*
	[19,22,25,69,73,74]	(Kim et al. 2015)	(Kim et al. 2015)	[18,20,69]	[39,68]	[39]
Enterobacter	+	+	+	-	+	-
Serratia	-	+	-	+	-	+
Arthrobacter	-	-	-	+	-	-
Microbacterium	+	+	-	+	+	-

Table 1: List of bacteria found in mosquito larvae (Guégan et al. 2018)

Enterobacter

Enterobacter is a commonly found bacteria genus in both the adult and larvae mosquito and in various mosquito species. Kim et al (2015) found *Enterobacter* in two different *Aedes* species larvae. They sampled two sites in rural Illinois, USA and looked at three different mosquito species. They collected water samples, fourth instar larvae, and pupa. Larvae were speciated through morphology and then dissected. The pupa were allowed to continue to develop and the emerged adult was also speciated by morphology and then dissected. They did show that the abundance of higher bacteria taxa was similar between the water samples and the larval gut microbiome as that there were significant differences between larval and adult midgut compositions. In total they found 57

culturable bacteria species between all water and mosquito samples. *Enterobacter* was also found in a *Culex* species.

Along with being a commonly found bacteria, there has also been evidence that *Enterobacter* specifically can play a role in vector competence, specifically inhibiting *Plasmodium* infection. Eappen et al (2013) found that AsSRPN6, an *A. stephensi* putative serine protease inhibitor is differentially induced by different bacteria. AsSRPN6 had been previously found to be expressed when midget cells had been infected by *Plasmodium* (Abraham et al. 2005). Eappen et al. found that AsSRPN6 was strongly expressed when exposed to gram-negative bacteria, whereas gram-positive bacteria elicited a weaker response. Cirimotich et al. (2011) that the *Enterobacter* species *Esp_Z* was able to inhibit the development of *Plasmodium* ookinetes without negatively affecting the mosquito fitness.

Serratia

Serratia has also been found to increase mosquitoes' susceptibility to dengue. *Serratia odorifera* specifically has been shown to enhance DENV-2 in *A. aegypti*. When *S. odorifera* was introduced to antibiotic treated *A. aegypti*, they showed a significant increase in their susceptibility to DENV-2 compared to a group of mosquitoes that did not receive additional bacteria and compared to a group that received a different bacteria species.

Serratia marcescens has been of particular interest due to its insecticidal effects. Prodigiosin is a metabolite alkaloid known for its bright red color. Its produced by the strain *S. marcescens* NMCC46. Patil et al. (2011) tested prodigiosin for larvicidal effects.

Testing it against *A. aegypti* and *A.* stephensi, they found that the mortality rate for all four instars positively increased with increasing dosage of prodigiosin. *S. marcescens* has also been found to limit *Plasmodium* infection in mosquitoes. Two *A. stephensi* treatment groups were challenged with *P. berghei*, one that had been cleared with antibiotics, another that had not. The group that had been cleared had a much higher infection rate. When *S. marcescens* was reintroduced to the cleared *A. stephensi*, it was able to inhibit *P. berghei*, but only when the mosquitoes were able to continuously feed on a *S. marcescens* solution as the mosquitoes cleared *S. marcescens* from their gut within three days (Bando et al. 2013).

Arthrobacter

The *Arthrobacter* genus are gram-stain-positive coryneform bacteria that have been found in a wide variety of environments. It is most commonly found in soils in different parts of the world. This genus is resistant to drying and starvation making it well adapted to soil environments, though their numbers decrease with increased soil acidity (Jones & Keddie 2006). *Arthrobacter* can also be found a range of insects. İnce et al. (2014) reported to isolate the species *Arthrobacter pityocampae* strain $Tp2^T$ from the pine processionary moth, *Thaumetopoea pityocampa*, a moth considered one of the most harmful pests of pine species in the Mediterranean. Moro et al. (2012) identified *Arthrobacter* as part of the microbiome of adult *A. albopictus* sampled from Madagascar.

Microbacterium

Microbacterium is part of the family Microbacteriaceae, a group of gram-positive primarily soil bacteria. *Microbacterium* itself has been isolated from various mosquitoes. Kim et al. (2015) were able to identify *Microbacterium* from unspeciated mosquito larvae sampled from rural Illinois.

Coon et al 2016 found that gnotobiotic reared *A. aegypti* and *A. atropalpus* that had been inoculated with *Microbacterium* all died as first instars. Similarly, in Coon 2016, the researchers attempted to rescue the development of axenic larvae by inoculating the breeding water with one type of bacteria. Of the seven different species, larvae inoculated with *Microbacterium* were one of two treatments that did not recover after it was added to the first instar larvae.

Bacteriophages

Bacteriophages are viruses that only infect bacteria. They play an important role in the evolution of functional and taxonomic diversity of the targeted host bacteria species through cell lysis, phage-bacterial co-evolution, and horizontal-gene transfer (Jasna et al. 2017, Touchon et al. 2017, Zeng et al. 2017, Paterson et al. 2010). This comes from two key factors in the bacteriophage life cycle. One is the bacteriophages' high specificity in infection. Most bacteriophages are so specialized that they can only target one species or one strain of bacteria. The second key factor is that bacteriophages have two distinct life cycles, a lytic and a lysogenic cycle. The lytic phase infects the bacteria cells, reproduces, and then lyses the cell to escape and continue propagation. In the lysogenic phase, the bacteriophage infects the bacteria cell, but then the bacteriophage's genetic material integrates into the bacteria's genome. Once integrated,

the bacteriophage "reproduces" and replicates as the bacteria replicates. Bacteriophages can be found almost anywhere bacteria are found (Wommack & Colwell, 2000). In recent years, bacteriophages have seen an increase in mainstream, clinical relevance as a potential solution to the rising problem of antibiotic resistant bacteria through the use of phage therapy, as a tool in food safety, and as a control in agriculture.

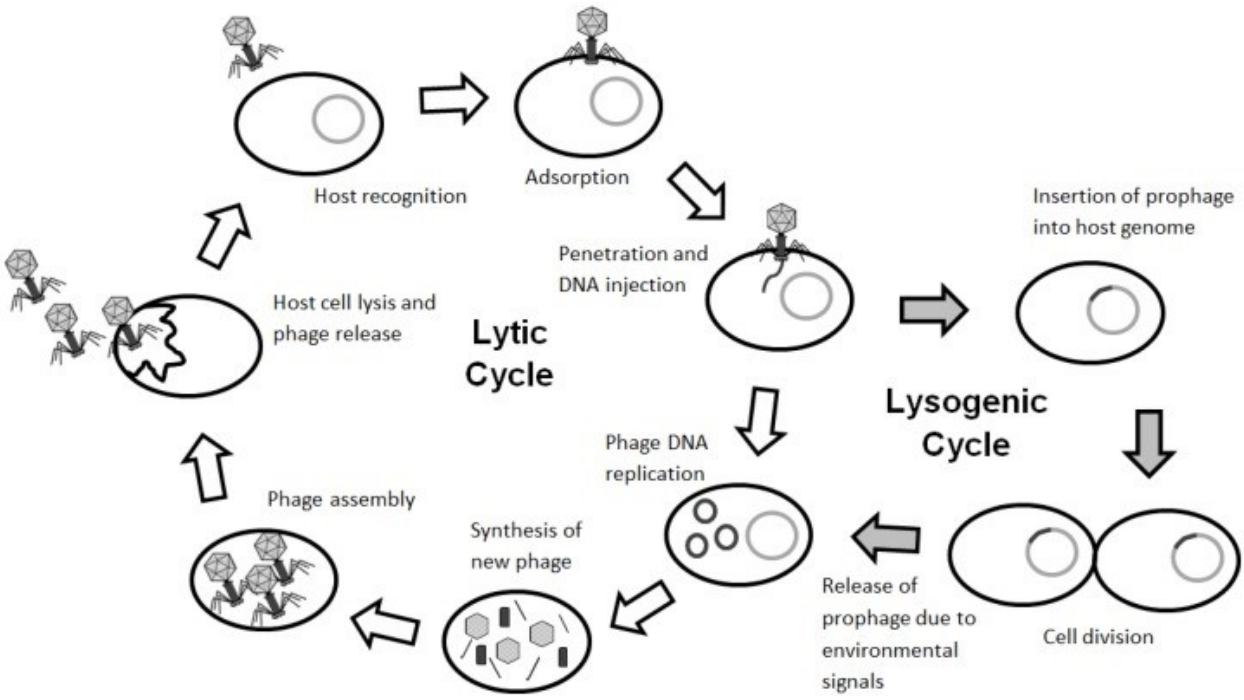

Figure 5: The bacteriophage lytic and lysogenic lifecycle (Doss et al. 2017)

Use of Bacteriophages *in Humans*

The first clear description of bacteriophages come from a French-Canadian scientist, Felix d'Herelle (d'Herelle 1917). What he observed were plaques forming from samples of filtered stool derived from dysentery patients. While initially searching for a possible virus cofactor in dysentery infection, d'Herelle found that the plaques, or the

bacteriophages, increased in titer as the disease progressed and were highest during recovery. He then inferred that there was “an exogenous agent of immunity” that assisted with recovery (Summers 2001). With this discovery, phage therapy was first applied to farm animals such as chickens and pigs to treat their infections. By the 1920s phage therapy had extended to human trials. The biggest breakthrough to the mainstream occurred when d’Herelle treated 4 patients infected with the bubonic plaque with a bacteriophage preparation and all 4 recovered quickly (d’Herelle 1926).

However, as standards started changing in the 1930s so did scrutiny for bacteriophage treatments and preparations. The actual biological nature of bacteriophages was still poorly understood; how did they clear the bacteria, how did they replicate, was there possibility for human infection; and there was no standard for potency or purity of the bacteriophage preparations. These two main factors made it difficult to assess bacteriophage treatments as different councils and associations attempted to standardize the pharmaceutical field (Summers 2001). Additionally, broad spectrum antibiotics began to pick up in popularity. Manufacturing became easier and these treatments were much more stable and easier to measure, both with regards to the results and the preparation.

However, phage therapy has continued to be a field of research and treatment in eastern Europe. This may have partly been due to the fact that in the 1930’s, the Soviet Union was at war with the Finns, and phage therapy was used to treat the wounded. Regardless of the historical reasons why phage therapy was never eclipsed by antibiotics in eastern Europe, phage therapies still remain a common and viable treatment there (Sulakvelidze et al. 2001, Summers 2001).

Recently, it has elicited interest the western world (Levin & Bull 2004). The increased risk and development of antibiotic-resistant pathogenic bacteria has pushed western scientists to look for alternative solutions (Burnham et al. 2018). As incidences of "superbug" infections continue to rise, the phage therapy treatments have begun to reach media headlines and have reignited interest in bacteriophages (Herschler 2016, Molteni 2019).

The phage-mediated therapeutic approach is currently a slow process. Bacteriophages have to be identified one by one in order to combat antibiotic-resistant bacteria. Most are found through screening processes sampling sewage water and other bacteria rich environments. Furthermore, for phage therapy to be effective or even work at all, the bacteriophages must be highly lytic. Instead of integrating into the bacteria genome, once the bacteriophage has infected the bacteria, it must start to reproduce and lyse the bacteria cells quickly. The more virulent the bacteriophage is and the more quickly it is able to reproduce, the more effective the treatment will be.

Additionally, bacteriophages have seen potential as antibody delivery and as diagnostic tools. Bacteriophages can be used to directly vaccinate if the bacteriophages are carrying the antigens on the surface of their coat or the bacteriophage can be used to deliver a DNA vaccine expression cassette that has been integrated into the bacteriophage's genome (Clark & March 2014). High specificity of bacteriophage infection of the host and inability to infect eukaryotic cells make them an ideal tool for selectively modifying any microbiota.

Use of Bacteriophages *in Agriculture*

Just like with human phage therapy, deliberate use of bacteriophages in agriculture goes back to the beginning of the 1900's. Mallman and Hamstreet observed in 1924 that filtering the liquid obtained from rotting cabbages and then applying it to growing fields of cabbages, it inhibited the growth of *Xanthomonas campestris pv campestris*, the bacterium that causes the rot. After that, numerous other scientists isolated bacteriophages to combat a number of plant pathogens, including *Pectobacterium carotovorum subsp atrosepticum*, the cause of the potato disease, blackleg disease (Kotila & Coons 1925); *Pantoea stewartia*, a bacterium that infects corn seeds (Thomas 1935); and *Xanthomonas pruni*, a bacterium that causes bacterial spots on peach seedlings (Civerolo & Keil 1969). However, a significant obstacle in bacteriophage treatments has been the actual application. A significant limiting factor has been the bacteriophages' ability to persist on the phyllosphere of plants, or the total above-ground portions. Laboratory and field experiments demonstrated that UV-A and UV-B rays were the most damaging to bacteriophage populations, but other factors such as rain and pH levels also contributed to lowering bacteriophage levels (Ignoffo et al. 1989, Ignoffo & Garcia 1992). Plant populations do tend to persist for longer when bacteriophages are applied to the rhizosphere (below ground portions of the plant). Bacteriophages stayed above detection levels one week after application if the roots were damaged and two weeks after application when the roots were healthy (Iriarte et al. 2012).

Bacteriophages have begun to be used commercially in the USA agriculture and food industry. In Baltimore, Intralytix Inc. is a company that sells aqueous bacteriophage preparations that target various food-borne illness causing bacteria such as *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella spp*. OmniLytics is another company

that has pursued research in bacteriophages' use in agriculture. Their product has been developed to control infections of tomato and pepper plants, apple and pear trees, and citrus trees. These precedents show the potential use and acceptance of bacteriophage-based interventions.

Bacteriophages in Insects

In the field of entomology, bacteriophages have been mostly studied within two areas: *Wolbachia* and aphids, both concerning lysogenic bacteriophages. *Wolbachia* was first described in 1924 by Hertig and Wolbach. Since its discovery, it has been estimated that *Wolbachia* can be found in at least 65% of insect species. *Wolbachia* has become of particular interest due to its ability to induce cytoplasmic incompatibility. Bacteriophage particles have been found in *Wolbachia's* genome and purified to reveal three prophage regions, a small pyocin-like element, *w*Mel WO-A, and *w*Mel WO-B (Wu et al. 2004). WO-B has gained particular interest due to its isometric head that contains linear double-stranded DNA and its ability to form mature virion particles. It is hypothesized that the WO-B may play a role in *Wolbachia's* famed cytoplasmic incompatibility effect (Fujii et al. 2004, Iturbe-Ormaetxe et al. 2005, Sinkins et al. 2005)

The pea aphid, *Acyrthosiphon pisum*¸ is preyed upon by the parasitic wasp, *Aphidius ervi*. However, when the pea aphid is inoculated with the bacterial symbiont, *Hamiltonella defensa,* the wasp eggs laid in the pea aphid fail to develop (Oliver et al. 2003). Unlike *Wolbachia*, *H. defens'*s prophage regions have been much better characterized. *H. defensa'*s prophages are referred to APSE. Thus far, three APSE variants have been characterized each encoding a different toxin gene; shiga tocin,

cytolethal distending toxin, and YD-repeat toxin (Moran et al 2005, Degnan & Moran 2008).

However, not all *H. defensa* infections contain APSE's and some *H. defensa* infections that do contain an APSE have been found to spontaneously lose that bacteriophage infection (Oliver et al. 2009). What has been observed, is that for *H. defensa* to be effective, APSE is required to be present in the bacterial genome (Oliver et al. 2009). Recently, it has been found that different strains of APSE have different effect on *H. defensa*'s infection and effect on aphids.

Recently, termites have also become an area of interest with regards to bacteriophages. Termites rely on their microbiota and the obligate nutritional mutualisms to assist in digesting plant matter (Bingell 2006). Kurtboke & French (2007) were interested in identifying and isolating known, but uncharacterized, gut symbionts of *Coptotermes lacteus*. They used bacteriophages to clear the gut of bacteria that they did know were colonizing the gut and were able to then reveal novel actinomycete species. Tikhe and Husseneder (2018) sequenced the metavirome of the *Coptotermes formosanus* termite to discover a varied community of known and unknown bacteriophages.

New Potential Uses of Bacteriophages

As bacteriophages can target specific bacteria, they can be used to affect the microbiome of the insect gut to affect vector capacity. In the unique lysogenic life cycle, bacteriophages could be used to potentially insert genes into bacteria that either produce toxins against pathogens in the microbiome, affect the mosquito's immune system to

lower vector capacity, or affect another part of the system in the spread of vector-borne diseases.

Lytic bacteriophages can target and lyse specific bacteria allowing for potential vector control uses to be handled more as a precise scalpel as opposed to the non-specific hammer that current insecticides are today. Research using lytic bacteriophages would also be able to look further into the purpose and effect of specific members of the mosquito's microbiome. As we learn more and more about how important the community of bacteria and other microorganisms are to the health and function of animals, we may be able to make useful applications of that knowledge. Already we have seen how some bacteria can have a direct effect with the vector capacity of mosquitoes as well as innate immune responses.

Bacteriophages appear to be an intriguing and novel tool to use for a better understanding of the mosquito microbiome. We have studied the utility of bacteriophages to modulate the mosquito microbiota in ways that will affect its lifecycle. We specifically investigated how the development of gnotobiotic mosquito larvae was affected when its microbiota was targeted with specific bacteriophages.

In order to better understand the mosquito microbiome, bacteriophages appear to be an intriguing and novel tool to use. We have conducted experiments where we investigate how mosquito larvae develop when placed in a gnotobiotic environments inoculated with one of three bacteria; *Escherichia coli* strain W3110, a well characterized strain*, Enterobacter spp*., and *Serratia spp*., two species isolated from lab-reared mosquito midguts. We then used the *E. coli* targeted bacteriophage T7, sourced from the company ATCC. The T7 bacteriophage is a commonly used and well characterized

bacteriophage that was discovered in 1945 by Demerec and Fano. Since then, it has been used extensively in the molecular biology field due to it encoding its own RNA polymerase and its ability to transcribe almost any sequence template with a T7 promoter (Sohn et al. 2003, Kutter & Guttman 2001).

MATERIALS AND METHODS

Mosquito Rearing

The Rockefeller strain of *Aedes aegypti* was reared at 27˚C with 80% humidity with a 12-hour light/dark cycle. Larval diet was made by mixing one part fish flakes, one part rabbit food, and two parts liver powder together and then grinding the mixture into a fine powder. Adult mosquitoes were maintained on a 10% sucrose solution.

Bacterial Cultures

10 ml of Lysogeny broth (LB) was inoculated with 10µl of bacterial freezer stock and was placed in a shaker overnight. *Enteroacter spp.* and *Serratia spp.* were all kept at 30˚C overnight. *E. coli* W3110 was kept at 37˚C overnight. The *E. coli* W3110 stock was obtained from ATCC (ATCC® 27325™). The *Enteroacter spp., Serratia spp., Microbacterium spp.,* and *Arthrobacter spp.* isolated had been previously isolated from dissections of lab reared mosquito midguts.

Before inoculating the larval breeding water with the bacterial liquid cultures, the cultures first had to be washed with Phosphate Buffer Solution (PBS). The PBS was made by dissolving prepared PBS tables in deionized (DI) water. The overnight bacterial culture was spun down in a centrifuge for ten minutes at 7300 rpm. The supernatant was poured off, 10 ml of PBS was added back to the tube, and the cells were resuspended. This was repeated twice more to ensure the LB was cleaned from the cells.

Agar Plates

Three different types of agar plates were used, a standard LB agar plate, a base agar plate, and a top agar plate. The LB agar plate was made with 20 g of LB powder and 15 g of agar all mixed in 1 L of DI water. The solution was then autoclaved and after autoclaving, about 10-15ml of molten LB agar was poured into plastic petri dishes. The base agar plates were made with 10 g of LB powder and 15 g of agar mixed with 1 L of DI water. The solution was then autoclaved and once cooled, 5 ml of 1M CaCl2 was added. 10-15 ml of molten base agar was then poured into petri dishes. The top agar plates were made with 20 g of LB powder and 3 g of agar mixed with 1 L of DI water. The solution was then autoclaved and once cooled, 5 ml of 1M CaCl2 was added. 5 ml of molten top agar would then be used to be poured onto of the base agar plates for the plaque assay.

Bacteriophage Isolation and Stock

The bacteriophage T7 was obtained from ATCC (ATCC® BAA-1025-B2™). Bacteriophages to target *Enteroacter spp.* and *Serratia spp.* were isolated from untreated waste water. Pre-treatment waste water samples were obtained from Baltimore Back River Wastewater Treatment Plant. 50 ml waste water samples were first centrifuged to remove large debris particles and then passed through a 0.2 µm filter (Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units) in order to remove the bacteria and smaller debris in the sample.

Plaque assays were then conducted. First, the filtered waste water was serially diluted in a 50:450µl ratio of waste water and phage buffer. Phage buffer was made by mixing and autoclaving 1 ml of 1M $MgSO_4$, 1 ml of 1M TrisCl, and 98 ml of DI water.

After cooled from the autoclave, 500 µl of 1M $CaCl_2$ was added and the entire solution was filtered through a 0.2 µm filter.

50 µl of a serial dilution was mixed with 450 µl of bacteria. A range of dilutions was used as the bacteriophage concentration was unknown, typically dilutions of 10^4, 10^6, and 10^8 were used. The serial dilution mixture was then incubated at either 30˚C or 37˚C depending on the bacteria used for 20 minutes. After incubation, the serial dilution mixture was mixed with 4.5ml of molten top agar and then was poured on a solidified base agar plate. These plates were then incubated for 24 hours at the respective temperature for the used bacteria. Plugs were taken from plates where clear, individual, isolated plaques were formed and then submerged in 1ml of phage buffer. If there were too many overlapping plaques and no isolated ones, the process had to be redone using a higher dilution. If no plaques formed at all, the process had to be repeated at a lower dilution.

After the plug had been sitting in the phage buffer for approximately 30 minutes, the phage buffer mixture was then passed through a 0.2 µm syringe filter and the plaque assay was repeated with the filtered phage buffer. This process was repeated a total for three times in order to purify a single bacteriophage type.

The final purified bacteriophage-phage-buffer solution was diluted to the point where, after conducting a plaque assay and being set overnight, plaques were too numerous and close together to be counted, but not at such a low dilution that the plaques covered the plate entirely. These were called web plates and once 10 web plates had been made and set overnight, approximately 5ml of phage buffer was used to cover the top of the plate. After sitting for approximately 30 min, the phage buffer was poured off and

passed through a 0.2 µm filter before being stored at 4°C. Bacteriophage concertation in these stocks were measured using plaque assay described earlier. These stocks were referred as high titer lysates.

Gnotobiotic Mosquito Assays

Mosquito eggs were surface sterilized using a 70% ethanol solution and a 3% bleach solution as described below. The ethanol solution, bleach solution, and sterile water were placed in a 6-well plate. Eggs were held in and moved with a cell strainer that fit into the wells. Eggs were submerged in the 70% ethanol solution for 5 minutes and then submerged in sterile water for 5 minutes. They were then submerged in the 3% bleach solution for 5 minutes and then sterile water for 5 minutes. The eggs were then again submerged in the ethanol solution for minutes before being submerged in sterile water 3 times, each for 5 minutes.

Once surface sterilized, the eggs were moved to a 200 ml cell culture flask with approximately 80 ml of sterilized water using a sterile cotton swab. The flask was then put into a vacuum for approximately 1-2 hours to force the eggs to hatch. Once hatched, 5-6 mosquito larvae were allocated into one well in a 6-well plate using a pipet.

The larval food solution was made at a concentration of 3.3 mg of larval diet per 100 µl of water and then autoclaved. The larvae were fed 3.3 mg of food on days 1 and 2. They were then fed 8.3 mg of food on days 4 and 5.

After mosquito larvae allocation, 500 µl of direct PBS washed bacterial overnight culture was added to each well and 500 µl of PBS was added to controls and sterile water was then added to the well until the total volume was at 5 ml. For the *Microbacterium*

spp., and *Arthrobacter spp.* experiments, 500 µl of direct PBS washed overnight liquid bacteria culture was added to one set of treatments. In another set of treatments, the washed culture was diluted by 10^4 through a serial dilution and added 500µl of that dilution was inoculated into the larval breeding water.

Because the mosquitoes must avoid contamination from both the other experiments with different bacteria and the bacteria present in the overall lab environment, the entirety of the gnotobiotic mosquito assay was performed in a laminar flow cabinet. Each unique gnotobiotic mosquito assay was replicated three times.

Single Time Bacteriophage Inoculation

Approximately 10^9 bacteria cells were added to the breeding water, which was about 500µl of direct PBS washed bacterial overnight culture measured at an OD600 reading of 1.4, along with approximately 10^9 of bacteriophage virions, 500 µl of bacteirophage high titer lysate, on day one. 100µl of sterile larval food solution was also added. For the controls, 500 µl of PBS and 500 µl of phage buffer were added to the wells. Sterile water was then added to the well until the total volume was at 5 ml. Once all treatments were set up in the 6-well plates, the plates were sealed and moved to an incubator where the larval were held in the environment described in the "Mosquito Rearing" section

Multiple Time Bacteriophage Inoculation

Similar to the Single Time Bacteriophage Inoculation protocol above, 10^9 bacteria cells and 10^9 bacteriophage virions were added to the breeding water (500 µl of direct

PBS washed bacterial overnight culture and 500µl of bacteriophage high titer lysate) on day one. 100 µl of sterile larval food solution was also added. For the controls, 500 µl of PBS and 500 µl of phage buffer were added to the wells. Sterile water was then added to the well until the total volume was at 5 ml. 10^9 bacteriophage virions (500 µl of bacteriophage high titer lysate) was then also added on day 2, 4, and 5, the same schedule as the feeding schedule. For the controls, 500 µl of phage buffer was added on days 2, 4, and 5.

Host Range

Once high titer lysates had been created, the host range of the isolated bacteriophages was tested. To do so, a bacterial agar plate was created. 500 µl of overnight bacterial culture was added to 4.5ml of molten top agar and the mixture was poured onto a solidified base agar plate and then set at room temperature. As the top agar set, bacteriophage serial dilutions were made. The high titer lysates were serial diluted in a 50:450 µl ratio of high titer lysate to phage buffer from 10^1 dilution to 10^6 dilution. Once the bacterial top agar had finished setting, sections were drawn on the plastic dish and each section was labeled from 10^1 to 10^6 with additional sections for a control and a direct sample. 10 µl of each serial dilution was then pipetted onto the top agar along with 10 µl of phage buffer for a negative control and 10µl of direct high titer lysate. The plates were left to set again at room temperature for 30-60 minutes. Afterwards, the plates were inverted and placed in a 30˚C or 37˚C incubator overnight. This assay demonstrated whether a bacteriophage could infect the bacteria it was tested against and if it does infect the bacteria, will give an estimate of the PFU/ml.

Culturing Bacterial Colonies and Bacteriophage Plaques

Each day, 50 µl of water samples were taken from each well to determine the Colony Forming Unit (CFU)/ml and the Plaque Forming Unit (PFU)/ml for that well. CFU/ml measurements were determined by serial diluting the water samples with PBS at a 50:450 µl ratio of water sample to PBS. The dilutions were then plated on LB agar plates, typically between the 10^4, 10^6, or 10^8 dilution. 500 µl of the bacteria dilution was then mixed with 4.5 ml of top agar and then poured onto base agar plates. The CFU/ml plates were then incubated at 30˚C or 37 ˚C overnight depending on the bacteria used.

PFU/ml measurements were similarly determined by filtering 100 µl of water samples through a 0.2 µm filter and then serial diluting those filtered samples at a 50:450µl ratio of water sample to phage buffer. 50 µl of the serial dilutions were then mixed with 450 µl of bacterial overnight stock and incubated at 30˚C or 37˚C for 15 minutes depending on the bacteria. After incubation, the bacteria and phage solution were mixed with 4.5ml of top agar and then poured onto base agar plates. The PFU/ml plates were then incubated at 30˚C or 37 ˚C overnight depending on the bacteria used.

Bacteriophage Growth Curves

300 µl of overnight bacterial culture was added to 30ml of liquid LB. The OD600 was then immediate taken for a time point of 0. After one-hour, high titer lysate bacteriophage was added to the tube. The amount of high titer lysate added to the 30 ml of bacterial culture depended on the chosen MOI for the bacteriophage that was being tested. The MOI was calculated as shown below:

$$MOI = \frac{PFU}{ml} / \frac{CFU}{ML}$$

A photometer was used to measure the OD600 and the OD600 was checked every hour for 6-8 hours. Phage buffer was added to a bacterial culture tube as a control the amount of phage buffer added matched the greatest amount of high titer lysate added to a treatment. Liquid LB was used as a blank. During the entirety of the growth curve experiment, the liquid culture tubes were kept on a shaker held at either 30˚C or 37˚C depending on the bacteria.

Bacteriophage Characterization and Genome Sequencing and Annotation

1 ml of the previously described high titer lysates was transferred to a 15 ml conical tube. 12.5 µl of 1 M $MgCl_2$, 0.4 µl of DNAase, and 1 µl of RNAase were then mixed into the tube and was left to incubate at room temperate for 30 minutes. Afterwards, 40 µl of 0.5 M EDTA, 5 µl of proteinase K, and 50 µl of 10% SDS were added in order, the tube was vortexed, and then incubated at 55°C for 60 minutes. During the 60-minute-incubation, the tube was vortexed every 20 minutes. After the incubation, the mixture was the processed using the standard DNA Phenol:chloroform:isoamyl alcohol at 25:24:1 extraction procedure. Once extracted, the DNA was run through on a gel electrophoresis to confirm successful extraction and then tested on the Thermo Scientific NanoDrop 2000 Spectrometer in order to determine the DNA concentration in the sample.

After extraction, the DNA was sent for sequencing using the Illumina MiSeq platform located at the Molecular Research MP in Shallowater, TX. The genomic reads were then assembled using the SPADES genome assembler available on the Cyverse

platform (http://www.cyverse.org/discovery-environment). Once assembled, the bacteriophage genome was analyzed using the GeneMark.hmm with Heuristic models gene prediction program using genetic code 11 (http://exon.gatech.edu/GeneMark/heuristic_gmhmmp.cgi, Besemer & Borodovsky 1999). Once the gene prediction were obtained, the NCBI BLASTp was used to manually annotate the genome for potential and hypothetical proteins. The bacteriophages' genomes were then compared visualized using the Easyfig (Sullivan et al. 2011) platform application.

High titer lysates were also sent to the Johns Hopkins Institute for Basic Biomedical Sciences Microscope Facility for bacteriophage visualization.

Statistical Analysis

All graphs were created in Microsoft Excel. Two sample t-tests (assuming unequal variances) were used to determine statistical differences between time points of different treatments. Unless otherwise stated, statistical significance refers to a p-value < 0.05.

RESULTS

Effect of the Presence of *E. coli, Enterobacter spp.,* and *Serratia spp.* in the Larval Breeding Water on Pupation

In order to evaluate any effects the bacteriophages had on mosquito larvae, we first had to create a baseline, by monitoring larval development under gnotobiotic conditions without bacteriophage exposure. We selected three bacteria species, *E. coli, Enterobacter spp.,* and *Serratia spp.* for this first round of tests. As seen in Figure 6, the *E. coli* and *Enterobacter spp.* appeared to influence larvae similarly with regards to pupation, development rates and survival. On day six, there was a significant difference between the percentage of pupation ($p<0.05$), however, that difference only lasted for one day. The median pupation time upon *E. coli* exposure was 5.65 days with 97% survival. With the *Enterobacter spp.* treatment, the media pupation time was 6.08 days with 98% survival. The *Serratia spp.* treatment delayed pupation by about two days compared to the other two treatments. The media pupation time was 7.88 days and the *Serratia spp.* treatment also resulted in a much lower pupa survival at 77%. There was a significant difference between the *Serratia spp.* treatment and the two other bacteria treatments starting on day six ($p< 0.05$). The larvae in the control wells did not develop and remained as first instar larvae.

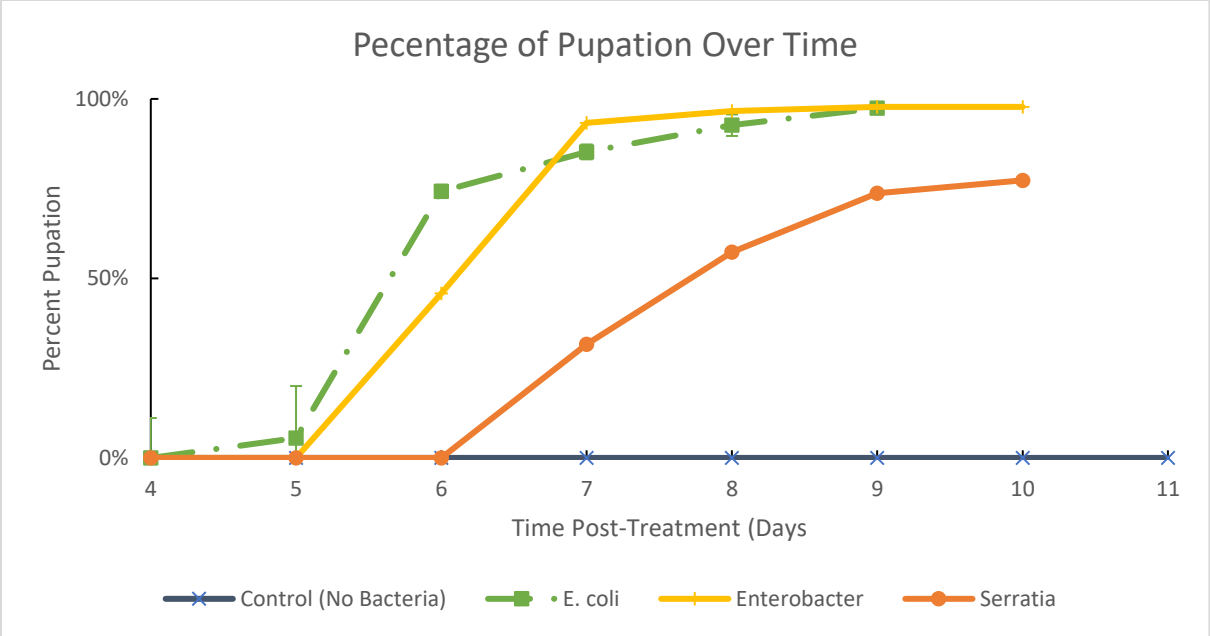

Figure 6: Breading water of gnotobiotic *A. aegypti* larvae were inoculated with *E. coli, Enterobacter spp.,* or *Serratia spp*.. Wells were checked daily for new pupa development and were monitored until all living larvae pupated (n=3). Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

Effect of Two *Microbacterium* Species and *Arthrobacter spp.* on Pupation

For the second round of pupation tests, we investigated the effect of two different *Microbacterium* species and *Arthrobacter spp*. when the larval breeding water was inoculated with either a direct sample from the overnight bacterial culture or a 10^4 dilution. Shown in Figure 7, we can see that *Microbacterium* 1 (MIB1) was not able to recuse larval development and neither the direct nor the diluted inoculations resulted in any pupation. *Microbacterium* 2 (MIB2) stimulated limited pupation and low survival. There was no significant difference between the direct or the diluted inoculations. The final percent of pupation upon MIB2-D inoculation was 38.9% and for MIB2-E4 it was 17.6%. *Arthrobacter spp.* (ARB) appeared to stimulate more standard development to pupation as well as increased survival compared to the two *Microbacterium* species; 68.8% for the direct inoculation and 81.3% for the dilution inoculation. While there was a significant difference between the direct and the diluted inoculations on day seven ($p < 0.05$), that difference disappeared by the next day. By day nine, the effects of both *Arthrobacter spp.* treatments were significantly different from that of the *Microbacterium* treatments. The larvae in the sterile control wells did not develop and stayed as first instar larvae.

Though in each replicate, each treatment has three replicate wells, only one full replicate has been done with this pupation test. Bacteria colony counts were not performed for this pupation test.

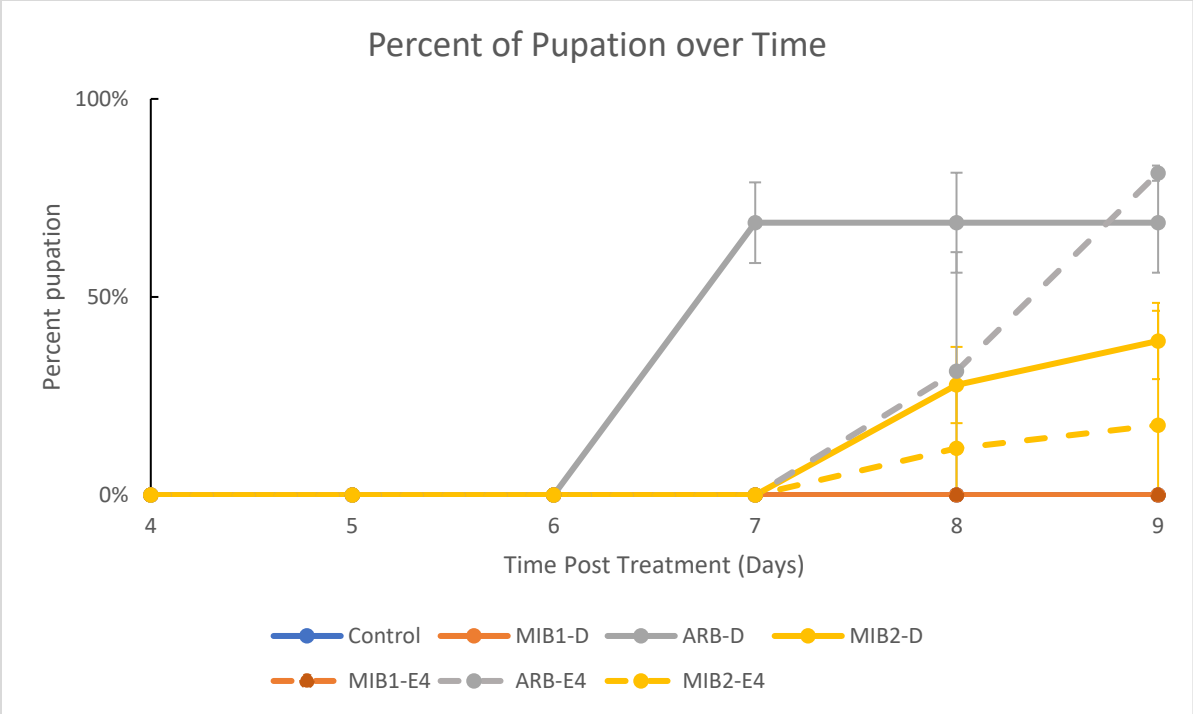

Figure 7: Breeding water of gnotobiotic *A. aegypti* larvae were inoculated with one of two different *Microbacterium* species (MIB1, MIB2) or *Arthrobacter spp.* (ARB). Two different concentrations were tested, either a direct inoculation (D, solid line) or an inoculation of a 10^4 (E4, dashed line) dilution. Wells were checked once a day for new pupa development and were monitored until all living larvae pupated (n=1). Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

E. coli, Enterobacter spp., and *Serratia spp.* Load in the Breeding Water

Bacterial load, measured by colony forming units per ml, were measured from water samples at the same time as the pupation measurements upon inoculations with *E. coli, Enterobacter spp*., or *Serratia spp*.. Only the first seven days of the treatment were measured as the majority of larval development occurred in this time period. By day seven, there were also some occurrences of emerging adults making it impossible to collect water samples. For the *E. coli* treatment, CFU/ml gradually decreased from 8.74 log(PFU/ml) on day one to 6.82 log(PFU/ml) on day four, at which the CFU/ml started to increase, coming back to 8.86 log(PFU/ml) by day seven (Figure 8). This was unlike either the *Enterobacter spp.,* or *Serratia spp.* measurements. In the *Enterobacter spp.,* and *Serratia spp*. treatments, the CFU/ml levels were more constant as compared to the *E. coli* measurements, with a slight increase in CFU/ml over time. *Serratia spp.* started at 6.31 log(PFU/ml) on day one, increased to 8.48 log(PFU/ml) by day three at which point increases of CFU/ml greatly slowed down (Figure 8). The *Enterobacter spp.* bacterial loads had more variability than those of *Serratia spp.*.

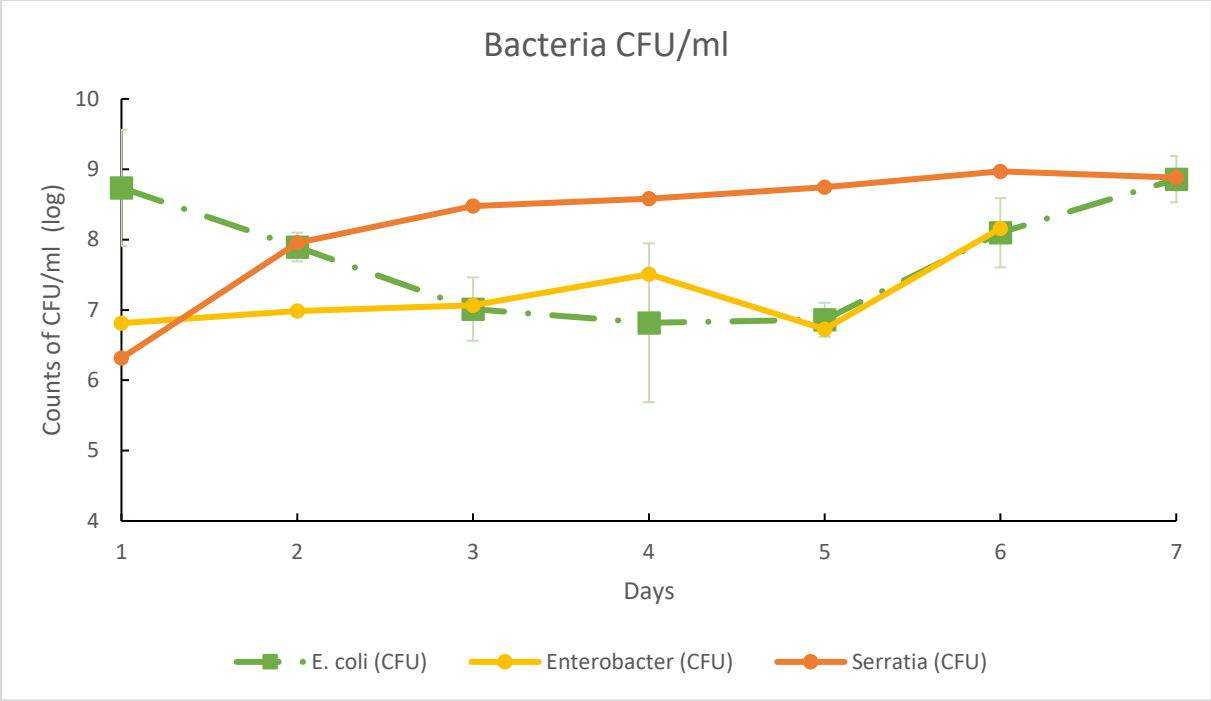

Figure 8: Breeding water of gnotobiotic *A. aegypti* larvae were inoculated with *E. coli, Enterobacter spp*., or *Serratia spp*. and water samples were taken from each well to measure colony forming units per ml (n=3). Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

Effect of T7 Bacteriophage-Mediated *E. coli* Lysis on Pupation

After establishing the percent pupation over time for the six bacteria, we used the T7 bacteriophage treatment to develop a model bacteria-bacteriophage system using *E. coli*. The T7 pupation experiment was carried out just as the other six bacteria-only treatments were, but 500µl of high titer bacteriophage lysate was added to the treatment wells at the start of the experiment on day one. When the bacteriophage lysate was only added on day 1, the median time of pupation in the *E. coli*+T7 bacteriophage treatment was 7.38 days and the percent survival was 81% (Figure 9). Compared to the median 5.65 days and a 97% survival for the *E. coli* treatment without bacteriophage, there was a clear different between the two treatments. Days six, seven, eight, and nine all had a significant difference between the *E. coli* and *E. coli*+T7 bacteriophage treatments ($p < 0.05$).

When the bacteriophage lysate was added on multiple days, there was a similar difference between the *E. coli*+T7 bacteriophage treatment and the control. As seen in Figure 10, as of day 8, the media time of pupation in the *E. coli*+T7 bacteriophage treatment was 5.59 and the percent survival was 90%. In the control, the median was 7 days and the percent survival was 61%. Due to contamination in the wells, the multiple bacteriophage inoculation experiment was not able to continue past day 8.

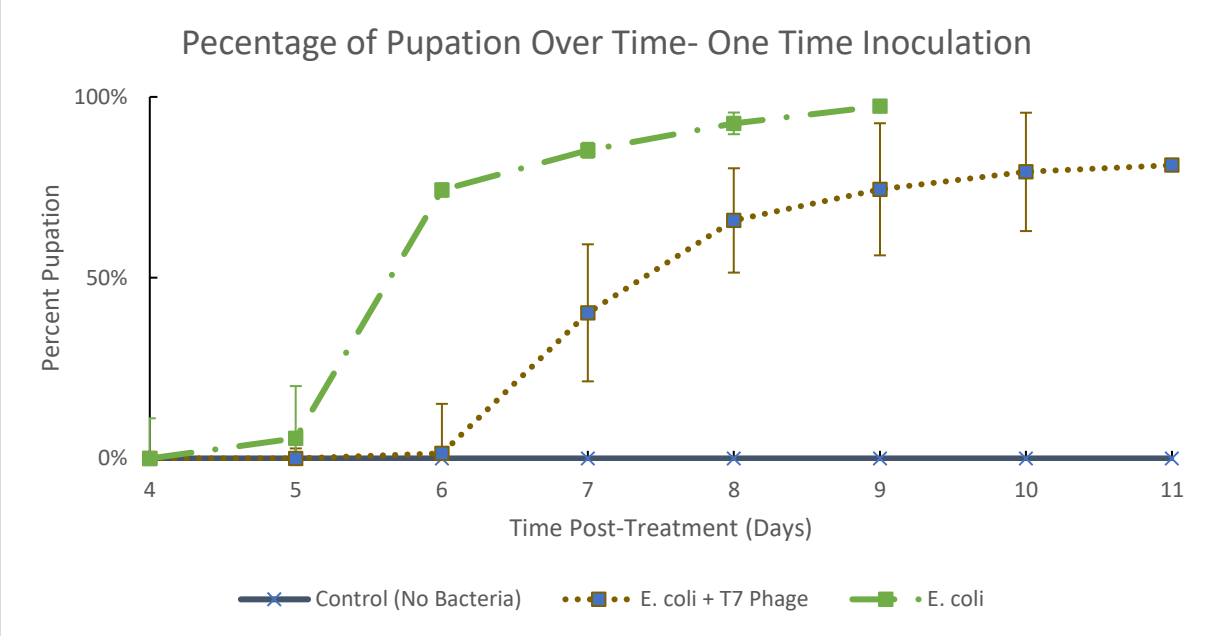

Figure 9: Breeding water of gnotobiotic *A. aegypti* larvae were inoculated with *E. coli*. One treatment was just inoculated with *E. coli* on day one, the other was inoculated with *E. coli* along with the T7 phage on day one. Wells were checked daily for new pupa development and were monitored until all living larvae pupated (n=3). Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

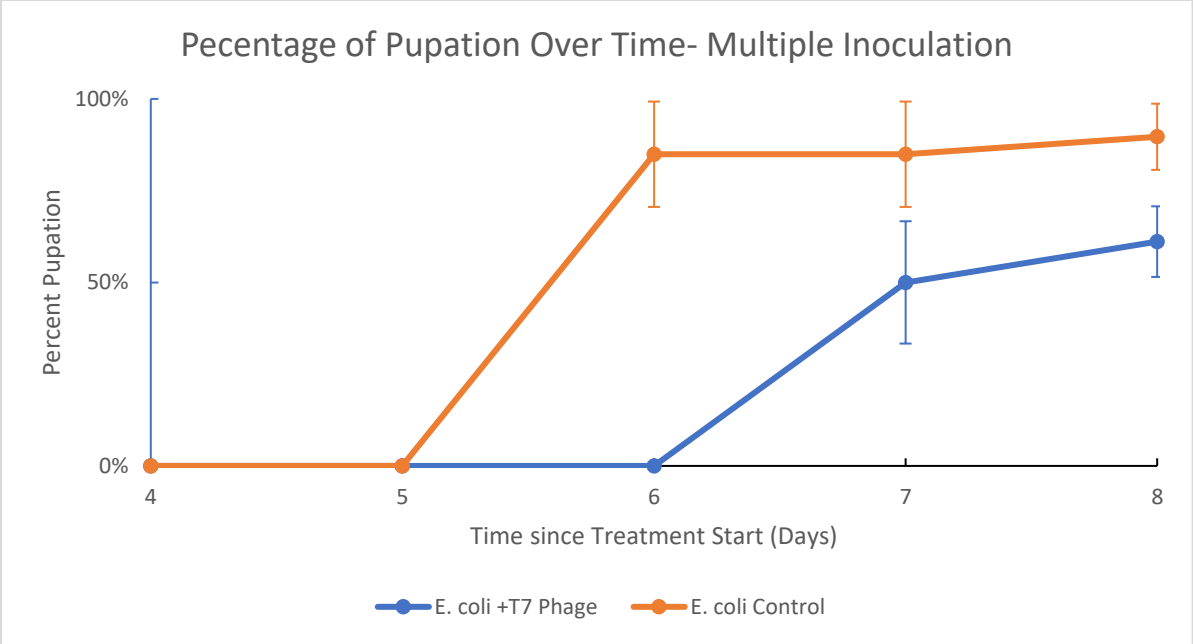

Figure 10: Breeding water of gnotobiotic *A. aegypti* larvae were inoculated with *E. coli*. In the *E. coli* +T7 bacteriophage treatment, bacteriophage was added on days 2, 4, and 5. Wells were checked daily for new pupa development and were monitored until all living larvae pupated (n=1). Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

Bacterial Load of *E. coli* in the Presence and Absence of T7 Bacteriophage in the Larval Breeding Water

There was a significant difference between the CFU/ml of the *E. coli* and *E. coli* +T7 bacteriophage one-time inoculation treatments on days two and three, but after day three, the *E. coli*+T7 bacteriophage treatment followed the *E. coli* CFU/ml trend and no significant differences were observed (Figure 11). The multiple-time inoculation treatments do not follow the same pattern as the one-time inoculation treatments. In the *E. coli* +T7 bacteriophage multiple-time inoculation treatment, there is a gradual decrease in the log(CFU/ml) from 7.83 on day one to 5.11 on day four. The log(CFU/ml) then recovers to 8.42 by day seven. It is in the *E. coli* control multiple-time inoculation treatment that the log(CFU/ml) drops on day two before recovering to similar day one levels for days 3, 4, 5, 6, and 7. There is a significant difference between the *E. coli* and *E. coli* +T7 bacteriophage one-time inoculation treatments on days two, three, and seven (Figure 12).

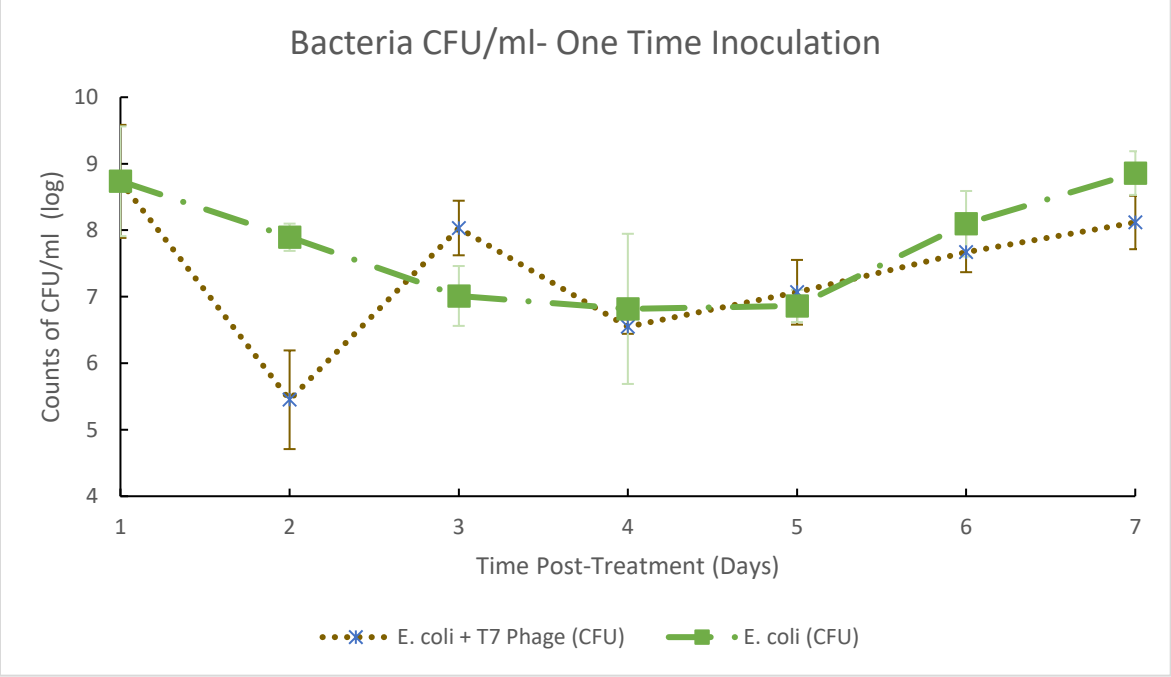

Figure 11: In the one-time bacteriophage inoculation *E. coli* treatments of the gnotobiotic *A. aegypti* larval breeding water, water samples were taken from each well to measure colony forming units per ml (n=3). Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

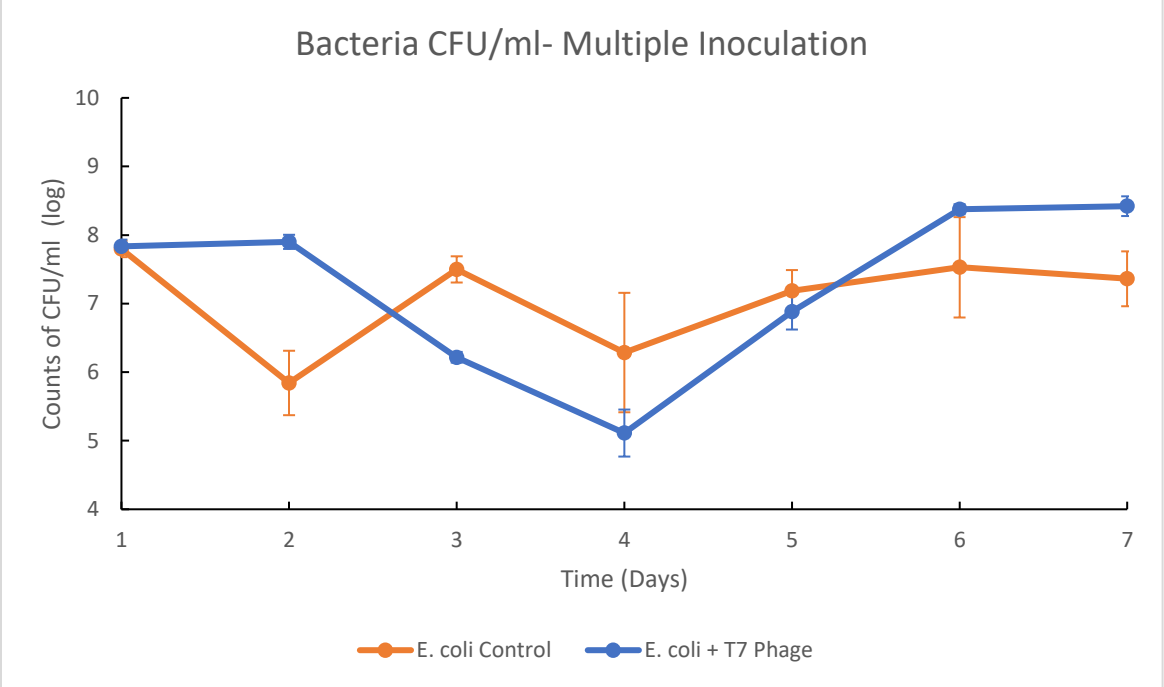

Figure 12: In the multiple-time bacteriophage inoculation *E. coli* treatments of the gnotobiotic *A. aegypti* larval breeding water, water samples were taken from each well to measure colony forming units per ml (n=1). Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

Bacteriophage Load of T7 in the Larval Breeding Water

Bacteriophage PFU/ml were also taken from the water samples of the *E. coli*+T7 bacteriophage treatments for the first seven days of the experiment. The drop from 8.85 log(PFU/ml) on day one to 6.78 log(PFU/ml) mirrors the drop in CFU/ml of the one time inoculation *E. coli*+T7 bacteriophage treatment. The T7 PFU/ml also climbs back up to similar levels as day one by the end of the seven days, similar to the pattern in the CFU/ml measurements of the treatment (Figure 13). The bacteriophage load of the multiple time inoculation treatment was not taken.

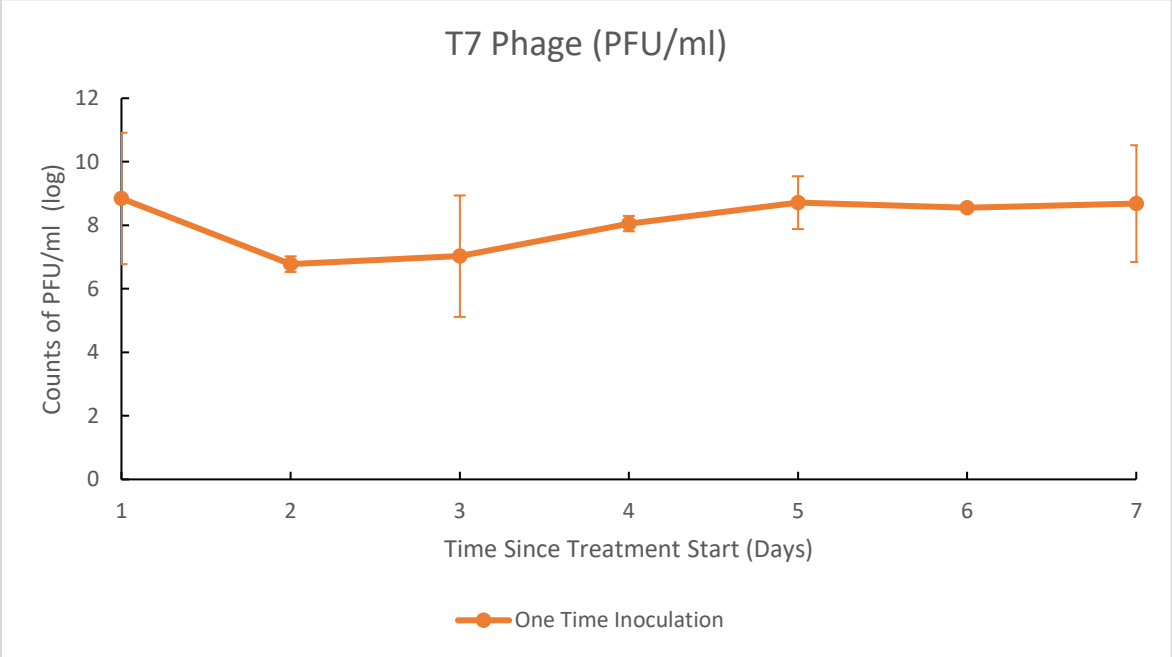

Figure 13: In both the one-time and multiple-time bacteriophage inoculation *E. coli* treatments of the gnotobiotic *A. aegypti* larval breeding water, plaque forming units per ml were measured from the *E. coli*+T7 bacteriophage treatments from water samples that were taken from each well (n=3). No difference between the two treatments was

observed. Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

Screening of Novel Bacteriophages

After establishing the *E. coli*+T7 bacteriophage model, we began using the untreated sewage water from the Baltimore Back River Wastewater Treatment Plant to screen for potential bacteriophages that targeted different bacteria strains. The bacteria and bacteriophage rich wastewater provide an ideal breed ground for a high density and diversity of bacteriophages (Tanji et al. 2008) allowing us to more easily collect potentially useful bacteriophages as opposed to using environmental samples. After filtering the wastewater, adding it to bacteria, and then preforming plaque assays, we were able to isolate eleven bacteriophages targeting *Enterobacter spp*. and two targeting *Serratia spp.*. However, there was no guarantee that we had isolated unique bacteriophages. As bacteriophages were obtained by identifying plaques as described in the methods, the only way of immediately identifying potentially different bacteriophages was through plaque morphology. Though there was a variety of plaque morphologies across the isolation assay, it was not guaranteed that all identified plaques were indeed due to unique bacteriophages. Isolating and sequencing the bacteriophage DNA would be the only method of confirming if all thirteen isolated bacteriophages were unique or if there were duplicates.

Testing the Host Range of *Enterobacter spp.* and *Serratia spp.* Bacteriophages

The EAP1 and EAP2 bacteriophages were isolated using *Enterobacter spp*. exposed to sewage water filtrate. To ensure that EAP1 and EAP2 only infected *Enterobacter spp.,* the two bacteriophages were challenged against five other bacteria that had been previously isolated from the mosquito midgut. Neither of the bacteriophages were able to infect and develop plaques on the other bacteria challenges besides *Enterobacter spp.* (Table 2). The host range assays for the SAP2 and SAP4 bacteriophages were established in the same way. SAP2 and SAP4 were isolated using *Serratia spp.* and like EAP1 and EAP2, only infected and created plaques with the bacteria they had been isolated from (Table 2).

		Mosquito Gut Bacteria					
		Entero	CSPP	Ser	Asaia	Elizab	Pseud
Bacteriophage	EAP1	O	X	X	X	X	X
	EAP2	O	X	X	X	X	X
	SAP2	X	X	O	X	X	X
	SAP4	X	X	O	X	X	X

Table 2: Host Range test of isolated bacteriophages. O indicates plaques did appear on the host range assay for that bacterial plate. X indicates that plaques did not appear on the range assay for that bacterial plate. The bacteria that the bacteriophages were challenged against were as follows: Entero- *Enterobacter spp*., CSPP- *Chromobacterium spp*., Ser- *Serratia spp*., Asaia- *Asaia spp*., Elizab- *Elizabethkingia spp*., Pseud- *Pseudomonas spp*.

Effect of Bacteriophage-Mediated *Enterobacter spp.* Lysis on Bacteria Load

Investigating the eleven *Enterobacter spp*. bacteriophages, we focused on two bacteriophages that had the strongest lytic ability, which was based on the morphology of the bacteriophage plaques. Bacteriophages that had largest and clearest plaques were chosen. Figure 14 shows that when those two bacteriophages tested (EAP1 and EAP2) against liquid *Enterobacter spp.* culture, both significantly lowered the OD600 reading compared to the control, indicating that both bacteriophages were able to effectively infect and lyse the bacteria ($p<0.05$).

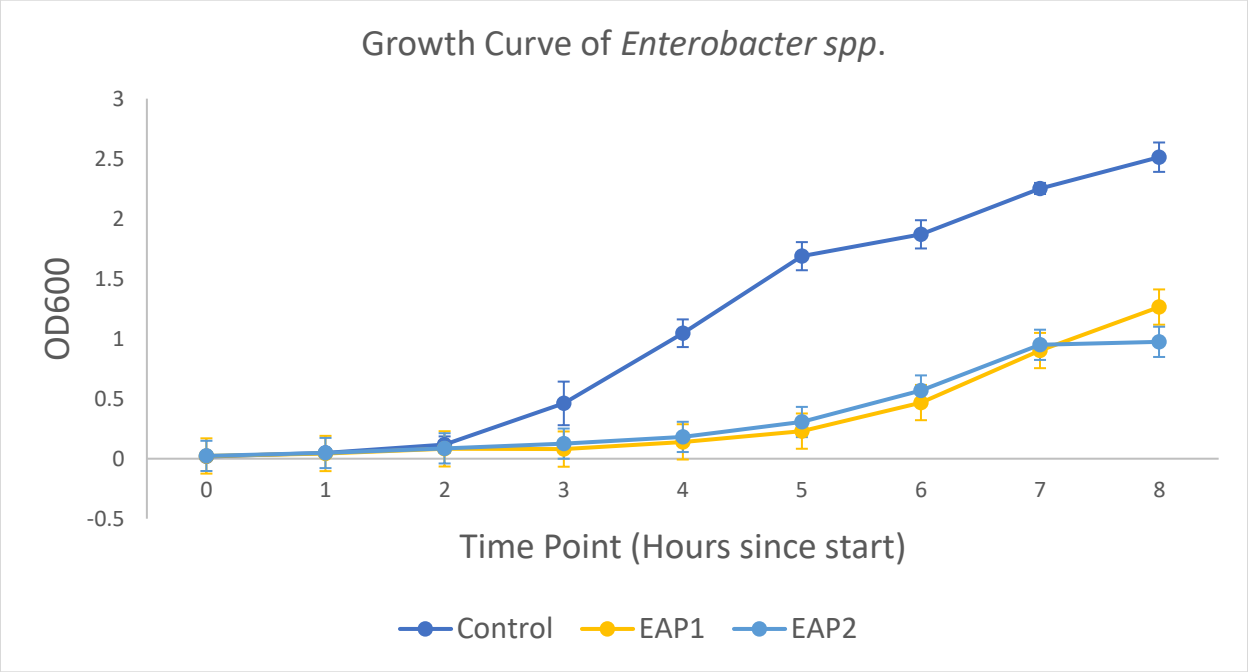

Figure 14: *Enterobacter spp.* growth curves depicting the effect of bacteriophage addition at an MOI of 10. Bacteriophage was added after the one-hour timepoint was taken. Both EAP1 and EAP2 had significantly different OD600 measurements as compared to the control by hour four. By hour eight, EAP1 and EAP2 had significantly different OD600 readings from the other (n=3). Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$).

Effect of Bacteriophage-mediated *Serratia spp.* Lysis on Bacteria Load

Serratia spp. bacteriophages were isolated and then tested in a *Serratia spp.* liquid culture challenge. Despite having developed plaques on a bacterial agar plate, it was clear that there was no effect of the bacteriophage on the *Serratia spp.* liquid culture. Both isolated bacteriophages, SAP2 and SAP4 were tested to evaluate their effect on their own and they were also combined to test of there might be any interaction between the two bacteriophages that would affect their infectiousness. None of the treatments show any significant difference from the *Serratia spp.* control as seen in Figure 15.

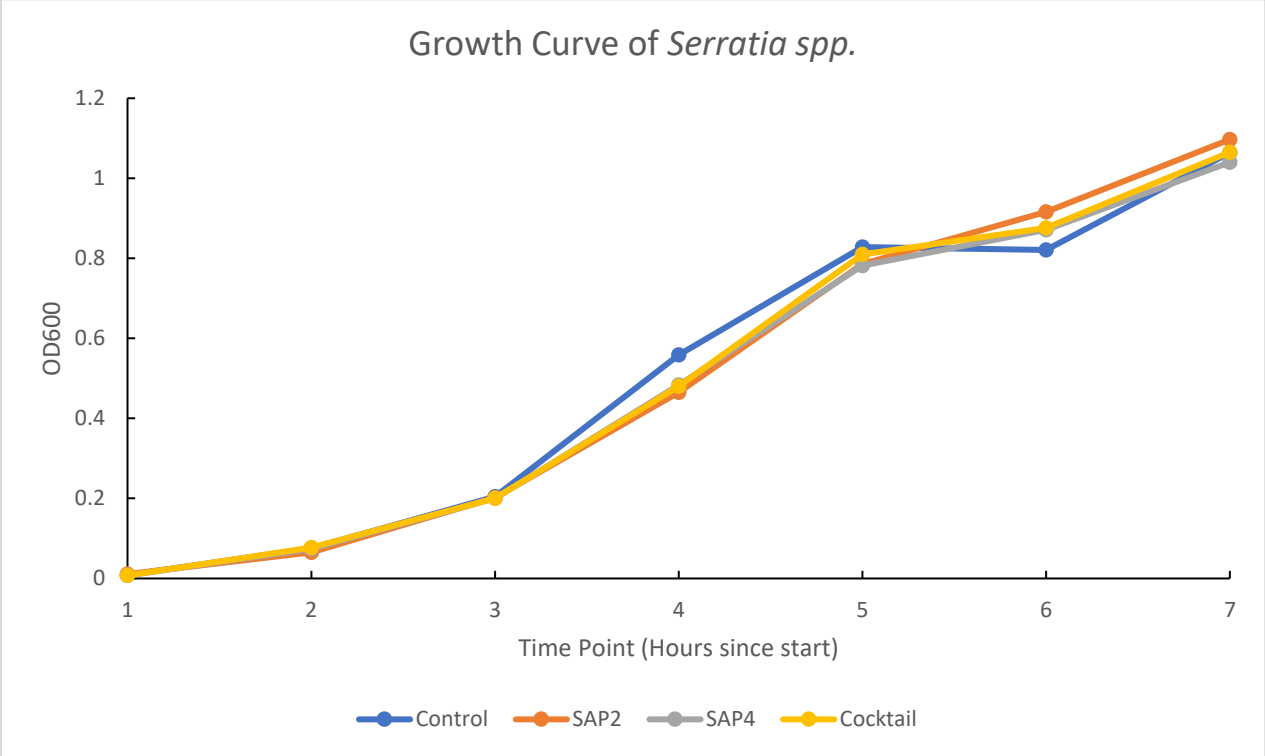

Figure 15: *Serratia spp.* growth curves depicting the effect of bacteriophage addition at an MOI of 100. Bacteriophage was added after the 1-hour timepoint was taken. There was no difference between the addition of either bacteriophage or the cocktail

combination of the two bacteriophages and the control. Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$) ($n=3$).

Effect of T7 Bacteriophage-mediated *E. coli* Lysis on Bacteria Load

Across the five tested bacteriophages, T7 shows the most drastic effect when inoculated with *E. coli*. Previous literature demonstrated that T7 was an extremely lytic bacteriophage and this data supports that conclusion. As shown in Figure 16, the bacteriophage takes immediate effect after it was inoculated into the bacteria liquid culture after the one-hour time point. By the two-hour time point, there was already a significant difference between the T7 and control treatments ($p < 0.05$).

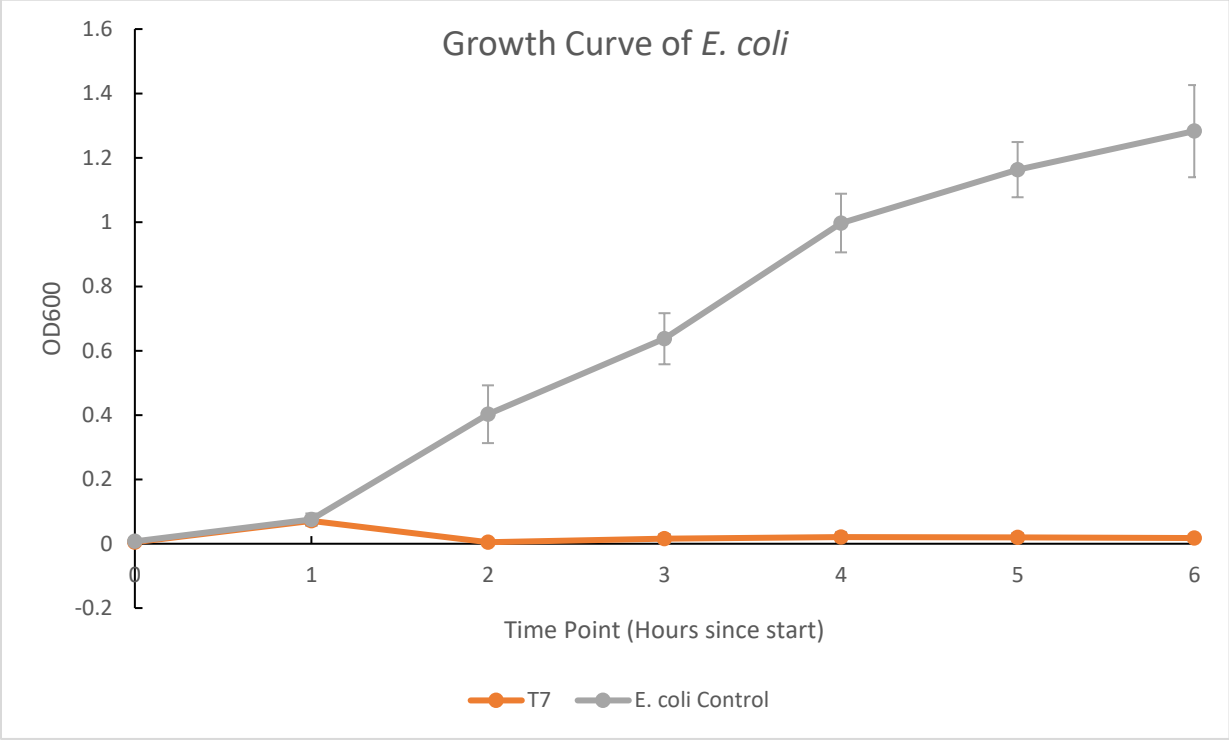

Figure 16: *E. coli* growth curve with a *E. coli* control and a T7 bacteriophage treatment. The bacteriophage was added after the one-hour timepoint was taken. By hour two, there was a significant difference between the two treatments throughout the assayed time-period. Two-sample t-tests were used to assess significant differences between different treatments' timepoints ($p<0.05$) ($n=3$).

Characterizing Enterobacter Bacteriophages

After isolating the two *Enterobacter spp.* bacteriophages, they were sent to be sequenced using the Illumina MiSeq platform, and then both bacteriophages were assembled and annotated (Supplemental Material 1 and 2). After manually annotating both genomes using the NCBI BLASTp database, we were able to see that their genome organizations were quite different with a low level of similarity which indicated that they are two separate bacteriophages (Figure 17). It was also revealed that EAP1 and EAP2 were not bacteriophages isolated from the sewage water, but instead prophages that had been integrated into the genome of an *Enterobacter* strain that had emerged upon exposure to the sewage water.

When first isolating bacteriophages from sewage water, the *Enterobacter* strain Esp_Z was used. Esp_Z has been previously sequenced and the sequence is publicly available on the NCBI GeneBank database. From there, it was known that there were four prophage regions in the genome, two were possibly viable, two were not. Initial attempts to stress Esp_Z to induce the lytic cycle of the prophages seemed to fail to induce bacteriophage production and it was assumed that all prophage regions were not viable, and experiments were carried out assuming that EAP1 and EAP2 were isolated from sewage water. However, it is clear that two of the regions were viable and that EAP1 and EAP2 had spontaneously induced from those regions.

Electron microscopy photographs were taken of both bacteriophages and from those photographs, both bacteriophages were able to be categorized by family based on their morphology. Both bacteriophages had clear elongated necks that are characteristic of the Myoviridae family (Figure 18 and Table 3).

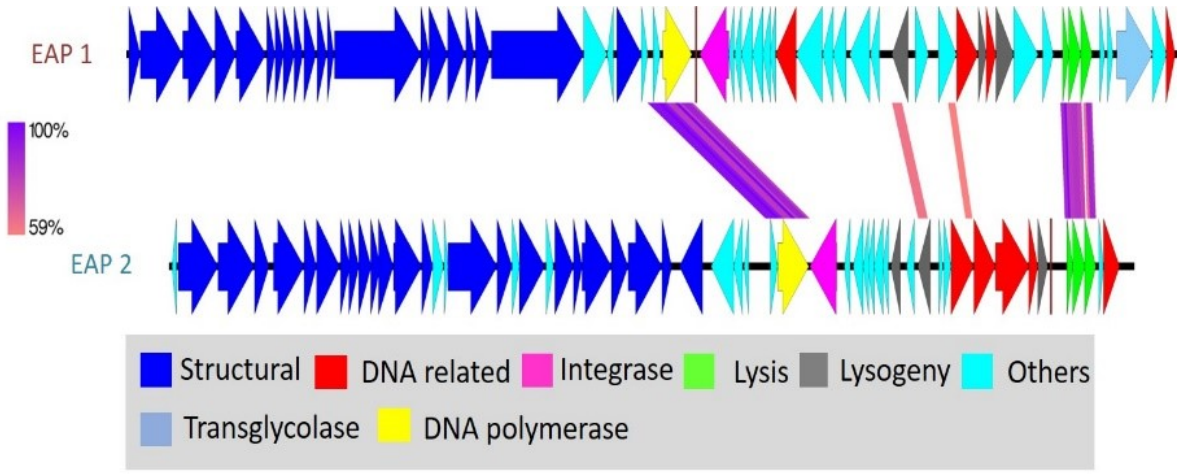

Figure 17: Genetic comparison of EAP1 and EAP2.

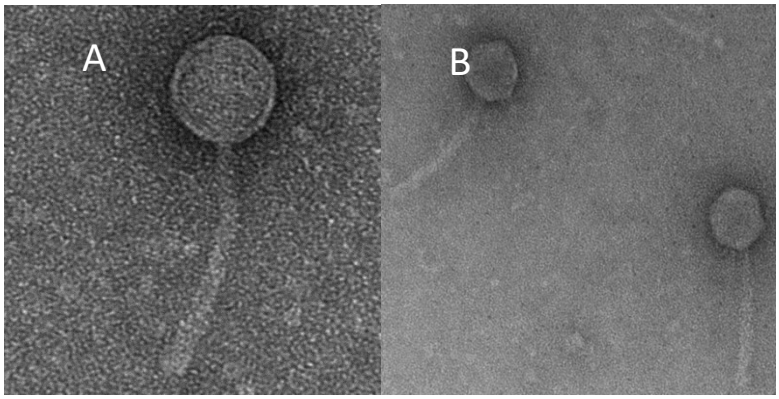

Figure 18: Electron microscopy photographs. A) Photograph of EAP1. B) Photograph of EAP2.

	Size	# genes	tRNA	Family
EAP 1	42,841 bp	56	Arg	Myoviridae
EAP 2	38,900 bp	54	Met	Myoviridae

Table 3: Genetic analysis of EAP1 and EAP2. Summary of the sizes of both bacteriophages and other characteristics, the start codon of the tRNA and the hypothesized family.

DISCUSSION

Mosquitoes have exerted an enormous public health burden on mankind for centuries due to their ability to transmit numerous viruses and other human pathogens. In order to combat their risk to humans, one of the most effective methods of control has been mosquito population control. Insecticides such as pyrethroids and organophosphates that target and reduce mosquito populations have been used globally. Pyrethroids, the most common adulticides, are used in control methods such as bed nets and indoor residual house spraying. They are popular due to their low toxicity in humans and their effectiveness in killing insects (Hougard et al. 2002). Organophosphates on the other hand are an example of larvicides. Resistance has been detected for both of these insecticides, along with the four other classes of insecticides (WHO 2019).

As resistance spreads, we will need to find new ways to control vector populations. The microbiota, being essential for the mosquito lifecycle, could be utilized for mosquito population control. However, new tools need to be developed to investigate the role of the microbiota, how it effects the mosquito lifecycle and vector competence.

In this study, we were interested in investigating how bacteriophages could modulate the larval breeding water microbiota, and how that could affect larval development. We first chose to inoculate sterile mosquito larval breeding water with one of six bacteria species, *E. coli, Enterobacter spp*., *Serratia spp*., one of two *Microbacterium* species, or *Arthrobacter spp.*. All the bacterium species except for *E. coli* had been isolated from the lab-reared *A. aegypti* mosquito microbiota. *E. coli* was chosen in order to have a bacteria species that was fully characterized and that had been previously shown to mimic similar larval development as non-sterile environments (Coon

et al. 2014). We used the W3110 strain (ATCC). Observing how the larvae developed when in the presence of just one bacteria species gave us insight on how different bacteria species could induce differences in development.

Of the six bacteria species, *E. coli* and *Enterobacter spp.* showed to have to have the fastest pupation rates and the highest survival rates. Larvae in the *Serratia spp.* and *Arthrobacter spp.* treatment groups took longer to pupate and displayed a lower pupal survival (Figure 6 and 7). The two *Microbacterium spp.* had very low pupation rates if the larvae even managed to pupate at all. *Microbacterium* species 1 was not able to rescue the larval development at all, regardless of how much bacteria was added. These clear differences between species were able to show that the type of bacteria added to the larval breeding water environment does matter. Coon et al. (2017) posited that larval development relied on bacteria-mediated hypoxia in the mosquito larval gut. This could indicate that the differences observed in larval development between bacteria species may indicate that the investigated bacteria species had different levels of oxygen usage and aerobic respiration. However, Correa et al. (2018) showed that live bacteria was not necessary for development and hypoxia may not be the only reason for continued development. Thus, differences in aerobic respiration may not be the only reason for differences of development. There may be differences in the proteins that the bacteria use or secrete or there may be a difference in how the mosquito immune system reacts to each bacteria species that affects development. Further research must be done in order to discover the molecular reason for the differences in development.

The colony forming units per ml (CFU/ml) was measured for the *E. coli, Enterobacter spp*., and *Serratia spp.* treatment groups over the course of the first seven

days of the experiments. The CFU/ml trends between the three species showed no clear pattern (Figure 8). In the *E. coli* treatment group, the CFU/ml decreased during the middle of the week before increasing again by day seven. *Enterobacter spp.* CFU/ml seemed to show a slow increase of over the course of the week. The *Serratia spp.* CFU/ml increased in the first two days before leveling off for the remaining time. It was unclear how the differences in CFU/ml trends affect the mosquito larvae. What was clear was that each of the three bacteria species demonstrate different influence on larval development. Not only was there an effect on pupation time and survival, but there was likely a difference in the bacteria concentration necessary for larval development.

After developing baseline larval development profile, we repeated the *E. coli* treatment and added the T7 bacteriophage to it. Addition of the bacteriophage resulted in a clear difference in pupation rate between the *E. coli* treatment and the *E. coli*+T7 bacteriophage treatment groups (Figure 9 and 10). With the T7 bacteriophage, pupation was delayed for over a day and the survival of the pupa also decreased. There were significant differences in the bacterial load (CFU/ml) only on day 2 and 3 (Figure 11) between the *E. coli* treatment and the *E. coli*+T7 bacteriophage treatment groups. On day 2, the CFU/ml of the *E. coli*+T7 bacteriophage treatment decreased significantly, but then rebound before falling back into the same trend as the *E. coli* treatment on day 4. The fact that the biggest difference in CFU/ml between the two treatments occurred on day 2 may imply that in order to best affect development, the early stages of the mosquito larvae should be targeted with phages. While the CFU/ml of the *E. coli*+T7 bacteriophage treatment group did recover to a similar level to the *E. coli* treatment group, the pupation rate and survival of the *E. coli*+T7 bacteriophage treatment group did

not. By using the *E. coli*+T7 bacteriophage system, we were able to demonstrate that bacteriophages can be used to affect the larval breeding environment and through that larval development.

However, the CFU/ml pattern in the *E. coli*+T7 bacteriophage treatment group was unexpected; when the T7 bacteriophage was added to the *E. coli* liquid culture, bacterial load not only dramatically decreased, but also stayed consistently low. In the gnotobiotic mosquito larvae assay, the CFU/ml decreased by day 2, but then increased and stayed at consistent levels afterwards. The discrepancy between the bacterial load in the liquid culture versus the larvae assay may be because the liquid cultures were kept on a shaker. The continued agitation increased the number of contacts bacteriophages had with potential host cells. In the larvae assay, the well plates were kept steady. Without the continued agitation, the number of contacts would have gone down. The discrepancy may also be due to the presence of the mosquito larvae. The experiment does not make it clear whether the larval development delay is due to a change in the breeding water environment or due to a change in the microbiome environment. If the bacteriophage is not able to persist in the mosquito larval midgut, the midgut, and thus digestive track could become a constant output of *E. coli* that is sufficient to keep the load in the breeding water constant.

Another explanation may be that the *E. coli* bacterial load did decrease and that the colonies observed were other bacteria that may have been introduced during the set up or duration of the experiment. While precautions were taken to ensure a complete gnotobiotic environment, contamination was still possible. Contamination was only determined by morphological identification of colonies. Thus, a bacteria species with

similar morphological traits may not have been noticed. Sequencing of the observed colonies may be a possibility in the future to confirm bacteria species.

We screened for bacteriophages that targeted *Enterobacter spp.* and *Serratia spp.*. After isolating several phages for each bacterium, we tested the effect of two bacteriophages, EAP1 and EAP2, against *Enterobacter spp.* in liquid culture. The two bacteriophages were able to drastically decrease the bacterial load as measured by an OD600 reading. By four hours, there was a significant difference between the bacteriophage treatments and the control. This difference persisted up to eight hours. Between EAP1 and EAP2, there was not an observed significant difference of their effect on the bacteria liquid culture. We also tested the effect of two more bacteriophages, SAP2 and SAP4, against *Serratia spp.* in liquid culture. These two bacteriophages did not have an effect on the bacteria growth, nor was there an affect when the two bacteriophages were combined. This indicates that both bacteriophages were in fact lysogenic and integrated themselves into the host cell genome. Further bacteriophage screening will be necessary in order to isolate other lytic bacteriophages that infect *Serratia spp..*

When EAP1 and EAP2 were sequenced, it was discovered that both were not new bacteriophages isolated from sewage water, but rather had been prophages, or bacteriophages that had been integrated to a host cell genome. When first isolating bacteriophages from sewage water, the *Enterobacter* strain Esp_Z was used. These isolated bacteriophages were then tested against a different *Enterobacter* species and was shown to infect that species (Table 2). We were very interested in seeing that these prophage regions were able to be spontaneously induce bacteriophage production.

Knowing that the Esp_Z genome has two viable prophage regions, makes the Esp_Z genome very interesting and generates the question of whether these prophage regions have an effect on the bacteria biology and if this then effects the mosquito midgut community. Previous research has shown that prophage regions can increase general fitness. Wang et al. (2010) found that when nine cryptic prophage regions were removed from *E. coli* K-12, there was a decrease in growth rate, increased sensitivity to antibiotics, and was not able to adapt to osmotic stress as well. Pursuing the possibility of developing Esp_Z non-prophage mutants and investigating how the Esp_Z prophage regions affect their host cell may help further understand the mosquito microbiome.

Spontaneous prophage induction is also not a well-studied subject; EAP1 and EAP2 may be useful in further investigating spontaneous induction itself. The two main theories for spontaneous induction is either stochasticity in host cell gene expression or the result of a stress-induced response (Nanda et al. 2015); though the mechanics have not been thoroughly investigated, there is interest in investigating how spontaneous induction affect the bacteria host beyond only lysing the host cell. It has been shown that prophages can affect biofilm development which in turn can also affect bacterial virulence (Nanda et al. 2015).

After obtaining the sequence for both bacteriophages, we then annotated both genomes. While many of the annotations were only hypothetical proteins, in both EAP1 and EAP2, there were similarities with APSE bacteriophage proteins (Supplemental Material 1 and 2). Considering the importance of the APSE bacteriophage in *H. defensa,* the presence of similar proteins provides information on hypothetical effects and influences EAP1 and EAP2 may have on the bacteria host genome.

From this study, we have been able to develop a bacteria-bacteriophage model using the *E. coli*+T7 bacteriophage system to study mosquito-bacteria-bacteriophage tripartite interactions. Through this system, we have been able to show that bacteriophages can affect the larval breeding water microbiota which has an effect on larval development.

Future Directions

The next immediate step would be to investigate an *Enterobacter spp.*-bacteriophage system along with a *Serratia spp.* one. We would then look at treatments where the bacteriophage was added to the larval breeding water at different time points. While our study did provide evidence that introduced bacteriophages will have the greatest effect on first-instar larvae, this may not always be practically possible. Hence, it is important to understand how larval development may be affected when bacteriophages are added to later stages of larval development.

We would also be interested in investigating the other bacteriophages that were isolated during the bacteriophage isolation assay. Only two of the eleven *Enterobacter spp.* targeting bacteriophages were tested in liquid bacteria culture. One or more of the other seven isolated bacteriophages may also show potential in being used in a *Enterobacter spp*.-bacteriophage system. Additionally, we would want to investigate other bacteria that have been isolated from the mosquito midgut such as *Pseudomonas spp., Asaia spp.,* and *Elizabethkingia spp.*. All of these bacteria species have been previously isolated and thus, additional bacteriophage screening would be required to isolate ones that targeted these bacteria.

Once several bacteria-bacteriophage systems have been developed, we would assess the combined effect of exposing larval breeding water to multiple phages. The mosquito microbiome comprises diverse communities of bacteria. In order to better model natural conditions, we have future plans to combine two or more bacterial cultures and then introduce one or multiple bacteriophages in order to assess how targeting one particular bacteria in a more diverse community may affect the mosquito larvae.

SUPPLEMENTAL MATERIAL

Supplemental Table 1

Gene #	Size/Strand/Position		Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
1	394_aa|-|189|1373	recombinase [Phage NG55]	548	548	98%	0	68.46 %	AZF93120.1
		integrase [Alteromonas phage P24]	249	249	98%	1.00E -77	39.85 %	AZU97334.1
		integrase [Marinobacter phage PS3]	157	157	98%	1.00E -42	31.76 %	ATN93328.1
2	71_aa|-|1375|1590	hypothetical protein [Phage NG55]	87	87	88%	7.00E -23	63.49 %	AZF93121.1
		putative YD-repeat toxin [Bacteriophage APSE-3]	31.6	31.6	57%	1.7	32.61 %	ACJ10121.1
		hypothetical protein SEA_RENAUD18_39 [Mycobacterium phage Renaud18]	28.9	28.9	64%	5.9	30.43 %	AXQ64949.1
3	89_aa|-|1615|1884	hypothetical protein mEp390_029 [Enterobacterial phage mEp390]	77	77	96%	3.00E -18	48.86 %	YP_007112446.1
		hypothetical protein [Klebsiella phage ST101-KPC2phi6.3]	60.1	60.1	94%	1.00E -11	43.02 %	QBQ71628.1
4	151_aa|-|1884|2339	hypothetical protein J46_0093 [Salmonella phage SJ46]	40.4	40.4	58%	0.011	30.70 %	YP_009293550.1
		EaA protein [Klebsiella phage 2 LV-2017]	38.1	38.1	31%	0.054	41.67 %	ARQ94722.1
		Eaa protein [Klebsiella phage 2b LV-2017]	35	35	81%	0.62	29.61 %	ARB15605.1
5	141_aa|-|2342|2767	hypothetical protein Sf101_0021 [Enterobacteria phage Sf101]	36.2	36.2	32%	0.2	41.38 %	YP_009153095.1
		Eae protein [Escherichia phage Rac-SA53]	35.4	35.4	20%	0.41	58.62 %	ALP46886.1
		hypothetical protein [Escherichia phage TL-2011c]	35	35	21%	0.55	56.67 %	YP_007001433.1
6	103_aa|-|2764|3075	hypothetical protein Ccr5_gp181c	36.6	36.6	84%	0.024	30.53 %	ARB14401.1

		[Caulobacter phage Ccr5]						
		hypothetical protein CcrRogue_gp185 [Caulobacter virus Rogue]	35	35	85%	0.1	32.29 %	YP_006989214.1
		hypothetical protein CcrMagneto_gp185 [Caulobacter virus Magneto]	34.7	34.7	92%	0.16	28.16 %	YP_006988867.1
7	73_aa|-|3065|3286	TraD/DksA family transcriptional regulator [Klebsiella phage ST16-OXA48phi5.2]	77.4	77.4	93%	6.00E-19	55.88 %	QBP28271.1
		hypothetical protein [Klebsiella phage 2 LV-2017]	75.5	75.5	93%	3.00E-18	55.88 %	ARQ94717.1
		TraR/DksA family transcriptional regulator [Klebsiella phage ST405-OXA48phi1.2]	75.1	75.1	93%	5.00E-18	54.41 %	QBP08160.1
8	278_aa|-|3283|4119	DNA adenine methyltransferase [Pseudomonas virus F116]	280	280	94%	9.00E-93	52.22 %	YP_164275.1
		DNA adenine methylase [Meiothermus phage MMP7]	261	261	94%	3.00E-85	50.19 %	AZF88245.1
		D12 class N6 adenine-specific DNA methyltransferase [Meiothermus phage MMP17]	260	260	94%	7.00E-85	50.19 %	QAY18084.1
9	356_aa|-|4116|5186	putative bacteriophage protein [Salmonella phage SPN9TCW]	515	515	96%	0	68.51 %	AFH20865.1
		DGQHR domain-containing protein [Klebsiella phage ST101-KPC2phi6.3]	494	494	99%	6.00E-175	66.67 %	QBQ71626.1
		unnamed protein product [Salmonella phage SPN1S]	491	491	91%	5.00E-174	69.14 %	YP_005098008.1
10	144_aa|-|5198|5632	hypothetical protein [Pectobacterium phage PEAT2]	90.9	90.9	59%	1.00E-22	48.84 %	ATV25116.1
		hypothetical protein [Escherichia phage TL-2011b]	63.2	63.2	81%	2.00E-11	36.62 %	YP_007001966.1

		hypothetical protein [Escherichia phage TL-2011c]	62	62	63%	9.00E-11	42.55 %	YP_007001433.1
11	180_aa|-|5623|6165	hypothetical protein sb35 [Salmonella phage ST64B]	264	264	98%	9.00E-90	76.84 %	NP_700408.1
		hypothetical protein [Escherichia phage 1720a-02]	241	241	99%	1.00E-80	62.57 %	AGR48387.1
		hypothetical protein SJJBTUD_0032 [Escherichia phage Ayreon]	240	240	98%	2.00E-80	63.28 %	ATE85503.1
12	|274_aa|-|6295|7119	hypothetical protein SfVp31 [Enterobacteria phage SfV]	378	378	100 %	9.00E-132	64.60 %	NP_599063.1
		hypothetical protein [Enterobacteria phage cdtI]	378	378	100 %	1.00E-131	64.96 %	YP_001272551.1
		hypothetical protein [Stx2-converting phage Stx2a_1447]	376	376	100 %	9.00E-131	64.60 %	BAT32385.1
13	120_aa|-|7156|7518	hypothetical protein sb37 [Salmonella phage ST64B]	179	179	94%	6.00E-58	72.57 %	NP_700410.1
		hypothetical protein 118970sal3_00049 [Salmonella phage 118970_sal3]	177	177	94%	3.00E-56	71.68 %	YP_009324760.1
		hypothetical protein SJJBTUD_0035 [Escherichia phage Ayreon]	145	145	96%	1.00E-44	59.48 %	ATE85514.1
14	219_aa|-|8030|8689	prophage repressor [Enterobacteria phage HK140]	283	283	96%	1.00E-95	63.01 %	YP_007111752.1
		prophage repressor [Escherichia phage mEpX2]	283	283	96%	2.00E-95	62.56 %	YP_007111479.1
		repressor protein cI [Salmonella virus HK620]	281	281	96%	5.00E-95	62.73 %	NP_112053.1
15	176_aa|+|8977|9507	hypothetical protein [Klebsiella phage 1 LV-2017]	263	263	86%	7.00E-90	84.31 %	ARB15775.1
		hypothetical protein [Klebsiella phage ST147-VIM1phi7.2]	261	261	86%	6.00E-89	83.66 %	QBP28458.1
		hypothetical protein mEp390_040 [Enterobacterial phage mEp390]	261	261	86%	7.00E-89	83.55 %	YP_007112457.1

16	245_aa|+|9919|10656	replication protein [Enterobacteria phage SfV]	287	287	98%	3.00E-96	56.62 %	NP_599071.1
		replication protein O [Stx2-converting phage Stx2a_1447]	287	287	98%	3.00E-96	56.62 %	BAT32392.1
		replication protein [Escherichia phage Ayreon]	286	286	98%	5.00E-96	56.62 %	ATE85491.1
17	292_aa|+|10656|11534	methyl-directed repair DNA adenine methylase [Salmonella phage 118970_sal3]	426	426	98%	5.00E-150	69.20 %	YP_009324772.1
		methyl-directed repair DNA adenine methylase [Salmonella phage SEN34]	426	426	93%	5.00E-150	73.90 %	YP_009191642.1
		Dam family site specific DNA-(adenine-N6)-methyltransferasase [Klebsiella phage ST101-KPC2phi6.3]	394	394	93%	7.00E-138	66.06 %	QBQ71614.1
18	108_aa|+|11544|11870	hypothetical protein SfVp42 [Enterobacteria phage SfV]	101	101	95%	2.00E-27	48.57 %	NP_599074.1
		DNA binding domain protein [Escherichia phage Ayreon]	101	101	95%	2.00E-27	48.57 %	ATE85518.1
		regulation protein [Shigella phage SfIV]	101	101	95%	2.00E-27	48.57 %	YP_008766907.1
19	129_aa|+|11867|12256	holliday-junction resolvase RusA [Enterobacterial phage mEp390]	224	224	96%	1.00E-75	83.87 %	YP_007112461.1
		holiday-junction resolvase [Salmonella phage 118970_sal3]	221	221	100 %	4.00E-74	79.84 %	YP_009324836.1
		holiday-junction resolvase [Salmonella phage ST64B]	219	219	99%	2.00E-73	79.69 %	NP_700418.1
20	239_aa|+|12268|12987	phage antirepressor protein [Cronobacter phage ENT47670]	263	263	88%	2.00E-87	61.01 %	YP_007237602.1
		DNA-binding protein [Cronobacter phage ES2]	263	263	88%	2.00E-87	61.01 %	AEM24700.1

		hypothetical protein [Siphoviridae sp.]	223	223	91%	9.00E-72	52.94 %	AXQ65850.1
21	328_aa|+|12990|13976	hypothetical protein mEp460_049 [Enterobacteria phage mEp460]	486	486	99%	2.00E-172	68.81 %	YP_007112123.1
		hypothetical protein SfII_47 [Shigella phage SfII]	485	485	99%	3.00E-172	68.50 %	YP_008318525.1
		hypothetical protein SfIV_45 [Shigella phage SfIV]	483	483	99%	2.00E-171	68.20 %	YP_008766909.1
22	142_aa|+|14166|14594	hypothetical protein ZF40_0025 [Pectobacterium phage ZF40]	243	243	99%	2.00E-81	79.43 %	YP_007006934.1
		hypothetical protein sb49 [Salmonella phage ST64B]	120	120	92%	3.00E-33	40.60 %	NP_700422.1
23	79_aa|+|14986|15225	hypothetical protein 7AX3_20 [uncultured Caudovirales phage]	103	103	97%	6.00E-29	75.32 %	ASN68757.1
		lysis S family protein [Klebsiella phage 2b LV-2017]	69.7	69.7	70%	8.00E-16	53.57 %	ARB15611.1
		holin [Enterobacteria phage phi80]	69.7	69.7	70%	9.00E-16	51.79 %	YP_007947980.1
24	167_aa|+|15225|15728	hypothetical protein 7AX3_21 [uncultured Caudovirales phage]	281	281	100 %	1.00E-96	76.65 %	ASN68758.1
		lysozyme [Klebsiella phage ST101-KPC2phi6.1]	201	201	94%	3.00E-65	60.62 %	QBP28384.1
		lysozyme [Klebsiella phage 2 LV-2017]	201	201	94%	3.00E-65	61.01 %	ARQ94697.1
25	154_aa|+|15718|16182	lysis protein [Klebsiella phage ST147-VIM1phi7.2]	208	208	98%	3.00E-68	65.56 %	QBP28447.1
		hypothetical protein 7AX3_22 [uncultured Caudovirales phage]	185	185	88%	5.00E-59	67.15 %	ASN68759.1
		endopeptidase [Enterobacteria phage phi80]	180	180	99%	3.00E-57	58.82 %	YP_007947982.1
26	73_aa|+|16197|16418	hypothetical protein 7AX3_23	102	102	98%	6.00E-29	62.50 %	ASN68760.1

		[uncultured Caudovirales phage]						
		hypothetical protein PPF1_21 [Rhizobium phage vB_RleM_PPF1]	71.6	71.6	100 %	1.00E-16	44.16 %	YP_009099584.1
		hypothetical protein AS1_14 [Marinobacter phage AS1]	57	57	95%	6.00E-11	41.43 %	AYP28994.1
27	66_aa|+|16496|16696	No significance						
28	76_aa|+|16806|17036	RNA polymerase-binding transcription factor [Pseudomonas phage PMBT3]	31.2	31.2	34%	2.4	42.31 %	AUM59658.1
29	485_aa|+|17192|18649	hypothetical protein [Aeromonas phage AsXd-1]	804	804	100 %	0	77.37 %	AXC33134.1
		hypothetical protein mEp390_054 [Enterobacterial phage mEp390]	803	803	100 %	0	78.40 %	YP_007112471.1
		glycosyl transferase [Cronobacter phage ENT39118]	798	798	100 %	0	77.98 %	YP_007238138.1
30	191_aa|+|18646|19221	hypothetical protein SP016_00190 [Salmonella phage FSL SP-016]	284	284	98%	6.00E-97	69.31 %	AGF88093.1
		hypothetical protein mEp390_056 [Enterobacterial phage mEp390]	273	273	99%	1.00E-92	70.53 %	YP_007112473.1
		hypothetical protein HK542_056 [Escherichia phage HK542]	261	261	100 %	7.00E-88	65.97 %	YP_007151791.1
31	121_aa|+|19206|19571	putative HNH endonuclease [Enterobacteria phage mEp235]	209	209	95%	5.00E-70	84.48 %	YP_007111637.1
		HNH endonuclease [Escherichia phage phi467]	187	187	95%	4.00E-61	73.91 %	CUW01228.1
		hypothetical protein P27p34 [Enterobacteria phage phiP27]	182	182	95%	3.00E-59	73.28 %	NP_543086.1
32	156_aa|+|19738|20208	small terminase subunit [Enterobacteria phage SfV]	197	197	98%	6.00E-64	62.03 %	NP_599033.1

		terminase small subunit [Salmonella phage ST64B]	196	196	98%	1.00E-63	62.66 %	NP_700374.1
		small terminase subunit [Shigella phage SfIV]	196	196	98%	2.00E-63	61.39 %	YP_008766865.1
33	576_aa|+|20205|21935	terminase large subunit [Klebsiella phage ST101-KPC2phi6.3]	1032	1032	98%	0	82.81 %	QBQ71599.1
		Terminase large subunit [Salmonella phage ST64B]	1019	1019	98%	0	83.22 %	NP_700375.1
		large terminase subunit [Shigella phage SfIV]	1013	1013	98%	0	82.51 %	YP_008766866.1
34	434_aa|+|21935|23239	portal protein [Enterobacteria phage mEp235]	774	774	100 %	0	84.56 %	YP_007111579.1
		portal protein [Klebsiella phage ST13-OXA48phi12.2]	728	728	100 %	0	79.77 %	QBP27574.1
		portal protein [Klebsiella phage ST147-VIM1phi7.2]	698	698	100 %	0	78.39 %	QBP28439.1
35	283_aa|+|23248|24099	Clp protease protein [Klebsiella phage ST13-OXA48phi12.2]	346	346	97%	1.00E-118	64.86 %	QBP27575.1
		Clp protease-like protein [Klebsiella phage ST147-VIM1phi7.2]	342	342	97%	2.00E-117	64.86 %	QBP28438.1
		head maturation protease [Enterobacteria phage mEp235]	330	330	97%	1.00E-112	63.41 %	YP_007111580.1
36	402_aa|+|24109|25317	major capsid protein [Klebsiella phage ST13-OXA48phi12.2]	622	622	99%	0	74.62 %	QBP27576.1
		major head subunit [Enterobacteria phage mEp235]	615	615	99%	0	74.12 %	YP_007111581.1
		major capsid protein [Klebsiella phage ST147-VIM1phi7.2]	610	610	99%	0	74.37 %	QBP28437.1
37	110_aa|+|25360|25692	head-tail connector II [Enterobacteria phage mEp235]	153	153	93%	5.00E-48	66.99 %	YP_007111583.1
		head-tail connector protein [Klebsiella	145	145	98%	1.00E-44	59.26 %	QBP27578.1

		phage ST13-OXA48phi12.2]						
		head-tail connector protein [Klebsiella phage ST147-VIM1phi7.2]	142	142	98%	1.00E-43	57.41%	QBP28435.1
38	112_aa|+|25701|26039	head-tail adaptor protein [Klebsiella phage ST13-OXA48phi12.2]	171	171	99%	7.00E-55	72.07%	QBP27579.1
		head-tail adaptor protein [Klebsiella phage ST101-KPC2phi6.3]	169	169	99%	4.00E-54	71.17%	QBQ71592.1
		head-tail adaptor protein [Klebsiella phage ST147-VIM1phi7.2]	164	164	99%	3.00E-52	69.37%	QBP28434.1
39	148_aa|+|26036|26482	hypothetical protein mEpX1_008 [Escherichia phage mEpX1]	213	213	100%	2.00E-70	67.79%	YP_007111645.1
		hypothetical protein HK446_008 [Escherichia phage HK446]	211	211	100%	1.00E-69	67.11%	YP_007111961.1
		hypothetical protein HK75_08 [Escherichia phage HK75]	210	210	100%	3.00E-69	66.44%	YP_004934115.1
40	115_aa|+|26479|26826	hypothetical protein [Aeromonas phage AsXd-1]	119	119	99%	2.00E-34	48.25%	AXC33173.1
		Gp11 [Escherichia virus HK97]	115	115	98%	8.00E-33	47.79%	NP_037705.1
		hypothetical protein mEp234_009 [Escherichia phage mEp234]	115	115	98%	1.00E-32	47.79%	YP_007112022.1
41	162_aa|+|26872|27360	major tail subunit [Enterobacterial phage mEp390]	251	251	95%	3.00E-85	76.62%	YP_007112427.1
		hypothetical protein ECP1_035 [Escherichia phage ECP1]	246	246	95%	4.00E-83	74.68%	ASJ79489.1
		major tail subunit [Escherichia phage HK542]	244	244	95%	2.00E-82	74.68%	YP_007151745.1
42	129_aa|+|27413|27802	tail assembly chaperone [Escherichia phage ECP1]	150	150	93%	4.00E-46	60.16%	ASJ79490.1
		tail assembly chaperone	149	149	93%	1.00E-45	60.16%	YP_007112428.1

		[Enterobacterial phage mEp390]						
		tail assembly chaperone [Escherichia phage HK542]	148	148	93%	2.00E-45	60.16 %	YP_007151746.1
43	87_aa|+|27826|28089	hypothetical protein mEp390_012 [Enterobacterial phage mEp390]	134	134	100 %	2.00E-41	72.41 %	YP_007112429.1
		hypothetical protein HK542_012 [Escherichia phage HK542]	133	133	100 %	8.00E-41	71.26 %	YP_007151747.1
		hypothetical protein [Morganella phage IME1369_02]	94.7	94.7	100 %	2.00E-25	51.72 %	ARM67967.1
44	1171_aa|+|28125|31640	putative tail length tapemeasure protein H [uncultured Caudovirales phage]	1189	1189	99%	0	59.52 %	ASN72481.1
		hypothetical protein [Aeromonas phage AsXd-1]	1171	1171	95%	0	60.79 %	AXC33168.1
		tail length tape-measure protein 1 [Klebsiella phage ST899-OXA48phi17.2]	1143	1143	94%	0	57.97 %	QBP28000.1
45	112_aa|+|31640|31978	gp17 [Escherichia virus HK022]	191	191	100 %	1.00E-62	77.68 %	NP_037677.1
		minor tail protein [Enterobacterial phage mEp390]	183	183	100 %	7.00E-60	73.21 %	YP_007112431.1
		minor tail protein [Escherichia phage HK75]	179	179	100 %	3.00E-58	71.43 %	YP_004934122.1
46	251_aa|+|31975|32730	gp18 [Escherichia virus HK022]	498	498	100 %	8.00E-180	94.02 %	NP_037678.1
		minor tail protein L [Enterobacteria phage HK140]	496	496	100 %	5.00E-179	93.63 %	YP_007111720.1
		gp18 [Escherichia virus N15]	452	452	100 %	7.00E-162	82.47 %	NP_046913.1
47	236_aa|+|32732|33442	minor tail protein [Enterobacterial phage mEp390]	478	478	100 %	1.00E-172	95.34 %	YP_007112433.1
		minor tail protein K [Escherichia phage mEp234]	474	474	99%	6.00E-171	94.89 %	YP_007112030.1
		minor tail protein [Escherichia phage mEpX1]	473	473	99%	1.00E-170	94.47 %	YP_007111654.1
48	111_aa|+|33473|33808	hypothetical protein mEpX2_019	60.5	60.5	100 %	3.00E-11	30.09 %	YP_007111455.1

		[Escherichia phage mEpX2]						
		hypothetical protein [Klebsiella phage ST846-OXA48phi9.1]	48.9	48.9	68%	8.00E-07	32.14 %	QBP07791.1
49	200_aa|+|33864|34466	tail assembly protein I [Enterobacteria phage mEp237]	292	292	100 %	3.00E-100	74.00 %	YP_007111392.1
		tail assembly protein I [Enterobacteria phage HK225]	282	282	98%	4.00E-96	74.49 %	YP_007112152.1
		tail assembly protein I [Escherichia phage mEp234]	281	281	100 %	1.00E-95	70.30 %	YP_007112032.1
50	1252_aa|+|34520|38278	central tail fiber [Escherichia phage HK633]	1842	1842	99%	0	73.61 %	YP_007112295.1
		phage host specificity protein [Escherichia phage HK75]	1833	1833	99%	0	73.37 %	YP_004934127.1
		central tail fiber J [Escherichia phage mEpX1]	1831	1831	99%	0	73.13 %	YP_007111657.1
51	317_aa|+|38278|39231	hypothetical protein Vid5_gp23 [Pantoea phage vB_PagS_Vid5]	207	207	99%	4.00E-63	39.14 %	AVJ51778.1
		hypothetical protein P13BB106kb_p108 [Pectobacterium phage DU_PP_V]	80.5	80.5	70%	7.00E-16	29.00 %	ATS94092.1
		hypothetical protein AU5Stx1_770 [Stx1 converting phage AU5Stx1]	50.8	50.8	35%	1.00E-05	29.17 %	ANJ63870.1
52	83_aa|-|39237|39488	phage superinfection exclusion protein Cor like protein [Stx2-converting phage 86]	48.5	48.5	85%	2.00E-07	29.58 %	YP_794070.1
		putative phage superinfection exclusion protein Cor-like protein [Salmonella phage SSU5]	48.1	48.1	85%	3.00E-07	30.99 %	YP_006906657.1
		gp32 [Enterobacteria phage ES18]	47	47	93%	8.00E-07	32.10 %	YP_224170.1

53	335_aa|+|39625|40632	hypothetical protein [Aeromonas phage AsXd-1]	283	283	54%	1.00E-91	79.57%	AXC33161.1
		tail fiber [Escherichia phage HK542]	184	184	52%	2.00E-52	54.80%	YP_007151758.1
		tail fiber [Escherichia phage HK544]	175	175	52%	2.00E-49	51.98%	YP_007151631.1
54	82_aa|+|40634|40882	No significance						
55	94_aa|+|41106|41390	hypothetical protein mEp390_026 [Enterobacterial phage mEp390]	68.6	68.6	92%	1.00E-14	40.23%	YP_007112443.1
		hypothetical protein [Klebsiella phage ST11-OXA245phi3.2]	61.2	61.2	62%	7.00E-12	50.00%	QBQ71778.1
		hypothetical protein [Klebsiella phage ST15-VIM1phi2.1]	57	57	81%	3.00E-10	41.56%	QBP28193.1
56	48_aa|+|41392|41538	translession error-prone DNA polymerase subunit [Klebsiella phage ST846-OXA48phi9.2]	68.9	68.9	83%	4.00E-14	77.50%	QBP07754.1
		putative error-prone lesion bypass DNA polymerase V [uncultured virus]	68.6	68.6	83%	5.00E-14	72.50%	ASF00803.1
		translesion error-prone DNA polymerase V subunit [Escherichia phage PHB10]	68.6	68.6	83%	5.00E-14	77.50%	QBJ00792.1
57	385_aa|+|41535|42692	translession error-prone DNA polymerase subunit [Klebsiella phage ST846-OXA48phi9.2]	612	612	98%	0	74.08%	QBP07754.1
		DNA polymerase [Cronobacter phage ENT39118]	602	602	99%	0	74.48%	YP_007238154.1
		translesion error-prone DNA polymerase V subunit [Escherichia phage PHB10]	597	597	99%	0	71.95%	QBJ00792.1

SP Table 1: EAP1 Annotation

Supplemental Table 2

Gene #	Size/Strand/Position		Max Score	Total	Query	E value	Per. Ident	**Accession**

				Score	Cover			
1	|393_aa|+|1|1182	translession error-prone DNA polymerase subunit [Klebsiella phage ST846-OXA48phi9.2]	622	622	98%	0	73.85 %	QBP07754.1
		DNA polymerase [Cronobacter phage ENT39118]	613	613	99%	0	74.62 %	YP_007238154.1
		translesion error-prone DNA polymerase V subunit [Escherichia phage PHB10]	608	608	99%	0	71.76 %	QBJ00792.1
2	363_aa|-|1232|2323	site-specific recombinase [Morganella phage IME1369_02]	430	430	98%	2.00E-149	57.22 %	ARM67955.1
		site-specific recombinase [Escherichia phage 1720a-02]	416	416	99%	1.00E-143	53.85 %	AGR48329.1
		integrase [Enterobacteria phage mEp460]	405	405	99%	9.00E-140	53.02 %	YP_007112096.1
3	85_aa|-|2619|2876	hypothetical protein [Morganella phage IME1369_02]	101	101	100 %	4.00E-28	52.94 %	ARM68013.1
		hypothetical protein [Escherichia phage 1720a-02]	101	101	88%	7.00E-28	64.00 %	AGR48395.1
		hypothetical protein mEp460_025 [Enterobacteria phage mEp460]	101	101	96%	7.00E-28	59.76 %	YP_007112099.1
4	137_aa|-|2979|3392	hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	183	183	99%	4.00E-59	64.03 %	QBP07854.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.3]	183	183	100 %	5.00E-59	63.57 %	QBP07971.1
		hypothetical protein KPP56652_31 [Klebsiella phage KPP5665-2]	176	176	100 %	4.00E-56	60.58 %	ASX98647.1
5	84_aa|-|3582|3836	hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	142	142	100 %	1.00E-44	82.14 %	QBP07856.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.3]	142	142	100 %	2.00E-44	80.95 %	QBP07973.1
		hypothetical protein KPP56652_33 [Klebsiella phage KPP5665-2]	142	142	100 %	2.00E-44	82.14 %	ATE82632.1

6	121_aa|-|3829|4194	hypothetical protein [Aeromonas phage AsXd-1]	207	207	92%	8.00E-69	92.86%	AXC33151.1
		hypothetical protein KPP56652_34 [Klebsiella phage KPP5665-2]	206	206	99%	2.00E-68	85.00%	ASX98649.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	204	204	99%	1.00E-67	84.17%	QBP07857.1
7	76_aa|-|4187|4417	hypothetical protein [Klebsiella phage ST16-OXA48phi5.3]	75.1	75.1	90%	5.00E-18	53.62%	QBP07975.1
		hypothetical protein STM0898.4n.Fels1 [Salmonella phage Fels-1]	36.6	36.6	76%	0.006	32.76%	YP_001700545.1
8	124_aa|-|4524|4898	hypothetical protein [Aeromonas phage AsXd-1]	179	179	97%	1.00E-57	68.80%	AXC33149.1
		putative phage repressor [Cronobacter phage ENT39118]	134	134	82%	7.00E-40	52.94%	YP_007238149.1
		helix-turn-helix transcriptional regulator [Phage NG54]	61.2	61.2	77%	2.00E-10	30.21%	AZF92952.1
9	169_aa|-|4961|5470	hypothetical protein [Swine acute diarrhea syndrome related coronavirus]	31.2	31.2	37%	7.6	28.12%	AVM80497.1
10	221_aa|-|5625|6290	prophage repressor [Enterobacterial phage mEp213]	216	216	59%	2.00E-69	74.05%	YP_007112395.1
		hypothetical protein 7AX3_1 [uncultured Caudovirales phage]	213	213	84%	4.00E-68	54.45%	ASN68745.1
		hypothetical protein ECP1_061 [Escherichia phage ECP1]	207	207	57%	3.00E-67	74.80%	ASJ79515.1
11	94_aa|+|6357|6641	prophage antirepressor [Enterobacteria phage mEp043 c-1]	85.5	85.5	77%	1.00E-21	50.68%	YP_007111551.1
		hypothetical protein 587AP2_57 [Mannheimia phage vB_MhS_587AP2]	69.3	69.3	77%	2.00E-15	45.21%	YP_009193608.1
		hypothetical protein jbd68_45 [Pseudomonas phage JBD68]	47.8	47.8	59%	2.00E-06	42.86%	ARM70507.1

12	98_aa|+|6675|6971	No Significance						
13	303_aa|+|6973|7884	hypothetical protein P27p17 [Enterobacteria phage phiP27]	447	447	96%	5.00E-158	74.83%	NP_543069.1
		hypothetical protein [Aeromonas phage AsXd-1]	195	195	93%	2.00E-59	45.10%	AXC33145.1
		replication protein O [Stx2-converting phage Stx2a_1447]	183	183	86%	1.00E-54	40.61%	BAT32392.1
14	292_aa|+|7901|8779	hypothetical protein [Aeromonas phage AsXd-1]	469	469	97%	3.00E-167	76.92%	AXC33144.1
		putative replication protein DnaC [Enterobacteria phage phiP27]	292	292	83%	7.00E-98	58.61%	NP_543070.1
		putative replication protein DnaC [Escherichia phage mEpX1]	226	226	96%	5.00E-71	43.05%	YP_007111684.1
15	458_aa|+|8776|10152	hypothetical protein [Aeromonas phage AsXd-1]	712	712	100%	0	76.25%	AXC33143.1
		putative helicase [Enterobacteria phage phiP27]	623	623	97%	0	66.29%	NP_543071.1
		replicative DNA helicase [Phage NG54]	352	352	97%	5.00E-116	42.60%	AZF92946.1
16	121_aa|+|10139|10504	RusA family crossover junction endodeoxyribonuclease [Klebsiella phage ST846-OXA48phi9.2]	205	205	95%	3.00E-68	81.74%	QBP07707.1
		RusA family crossover junction endodeoxyribonuclease [Klebsiella phage ST974-OXA48phi18.2]	177	177	96%	3.00E-57	65.81%	QBP28625.1
		RusA family crossover junction endodeoxyribonuclease [Phage NV21]	156	156	96%	1.00E-48	56.41%	AZF93082.1
17	134_aa|+|10504|10908	antitermination protein [Klebsiella phage ST13-OXA48phi12.2]	191	191	99%	4.00E-62	69.92%	QBP27560.1
		antitermination protein Q [Escherichia phage 1720a-02]	121	121	94%	8.00E-35	48.03%	AGR48371.1

		Q protein [Bacteriophage APSE-5]	117	117	90%	5.00E-33	47.93 %	ACJ10076.1
18	79_aa|+|11689|11928	hypothetical protein 7AX3_20 [uncultured Caudovirales phage]	103	103	97%	6.00E-29	75.32 %	ASN68757.1
		lysis S family protein [Klebsiella phage 2b LV-2017]	69.7	69.7	70%	8.00E-16	53.57 %	ARB15611.1
		holin [Enterobacteria phage phi80]	69.7	69.7	70%	9.00E-16	51.79 %	YP_007947980.1
19	167_aa|+|11928|12431	hypothetical protein 7AX3_21 [uncultured Caudovirales phage]	276	276	100 %	1.00E-94	74.25 %	ASN68758.1
		lysozyme [Enterobacteria phage phi80]	200	200	94%	8.00E-65	60.38 %	YP_007947981.1
		lysozyme [Klebsiella phage 2 LV-2017]	198	198	94%	4.00E-64	59.12 %	ARQ94697.1
20	150_aa|+|12421|12873	lysis protein [Klebsiella phage ST147-VIM1phi7.2]	184	184	88%	1.00E-58	67.14 %	QBP28447.1
		hypothetical protein 7AX3_22 [uncultured Caudovirales phage]	158	158	90%	1.00E-48	62.22 %	ASN68759.1
		endopeptidase [Enterobacteria phage phi80]	137	137	76%	3.00E-40	61.74 %	YP_007947982.1
21	61_aa|+|12997|13182	hypothetical protein PBI_SCTP2_125 [Salicola phage SCTP-2]	28.9	28.9	77%	8.6	27.66 %	ASV44140.1
22	218_aa|+|13186|13842	putative transposase [Cronobacter phage ENT47670]	345	345	93%	1.00E-120	76.96 %	YP_007237603.1
		putative transposase [Pectobacterium phage ZF40]	311	311	93%	8.00E-107	70.73 %	YP_007006948.1
		hypothetical protein [Klebsiella phage ST899-OXA48phi17.1]	302	302	93%	2.00E-103	67.16 %	QBP28545.1
23	157_aa|+|13874|14347	hypothetical protein AMBK_01 [Salmonella phage vB_SosS_Oslo]	232	232	84%	1.00E-77	81.20 %	YP_006560807.1
		hypothetical protein ZF40_0040 [Pectobacterium phage ZF40]	229	229	86%	2.00E-76	76.47 %	YP_007006949.1
		hypothetical protein [Klebsiella phage	216	216	100 %	3.00E-71	66.24 %	QBP28546.1

		ST899-OXA48phi17.1]						
24	556_aa|+|14756|16426	terminase large subunit [Salmonella phage SEN34]	956	956	96%	0	83.46 %	YP_009191608.1
		hypothetical protein [Phage NG54]	927	927	96%	0	81.56 %	AZF92929.1
		putative TerL [Burkholderia phage Bups phi1]	706	706	96%	0	62.13 %	ABY40529.1
25	490_aa|+|16429|17901	putative portal protein [uncultured Caudovirales phage]	926	926	100 %	0	89.80 %	ASN67475.1
		putative portal protein [Salmonella phage SEN34]	573	573	97%	0	58.04 %	YP_009191609.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	545	545	97%	0	55.35 %	QBP28314.1
26	196_aa|+|17918|18508	putative head morphogenesis protein [uncultured Caudovirales phage]	359	359	99%	9.00E-127	86.15 %	ASN67474.1
		hypothetical protein 7AX3_29 [uncultured Caudovirales phage]	359	359	99%	2.00E-126	86.15 %	ASN68766.1
		putative head morphogenesis protein [Salmonella phage SEN34]	236	236	99%	4.00E-78	55.61 %	YP_009191610.1
27	413_aa|+|18684|19925	putative head protein [uncultured Caudovirales phage]	704	704	100 %	0	80.39 %	ASN67473.1
		putative head protein [uncultured Caudovirales phage]	553	553	80%	0	79.15 %	ASN67700.1
		hypothetical protein SEN34_5 [Salmonella phage SEN34]	340	340	99%	2.00E-112	47.25 %	YP_009191611.1
28	157_aa|+|19957|20430	hypothetical protein 8AX10_2 [uncultured Caudovirales phage]	286	286	100 %	5.00E-99	91.08 %	ASN67472.1
		hypothetical protein SEN34_6 [Salmonella phage SEN34]	203	203	100 %	2.00E-66	64.56 %	YP_009191612.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	192	192	100 %	6.00E-62	60.51 %	QBP07879.1
29	312_aa|+|20445|21383	hypothetical protein 7AX3_32	569	569	100 %	0	86.86 %	ASN68770.1

		[uncultured Caudovirales phage]						
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	503	503	99%	8.00E-180	78.14 %	QBP07880.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	501	501	99%	3.00E-179	78.46 %	QBP28319.1
30	124_aa|+|21421|21795	hypothetical protein 7AX3_33 [uncultured Caudovirales phage]	163	163	100 %	2.00E-51	66.67 %	ASN68771.1
		hypothetical protein SEN34_8 [Salmonella phage SEN34]	81.6	81.6	96%	4.00E-19	40.98 %	YP_009191614.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	56.2	56.2	96%	1.00E-09	35.54 %	QBP28320.1
31	134_aa|+|21761|22165	hypothetical protein 7AX3_34 [uncultured Caudovirales phage]	227	227	100 %	2.00E-76	88.81 %	ASN68772.1
		hypothetical protein SEN34_9 [Salmonella phage SEN34]	183	183	100 %	4.00E-59	65.93 %	YP_009191615.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	166	166	100 %	5.00E-52	59.26 %	QBP28321.1
32	167_aa|+|22162|22665	hypothetical protein 7AX3_35 [uncultured Caudovirales phage]	288	288	100 %	1.00E-99	83.83 %	ASN68773.1
		hypothetical protein SEN34_10 [Salmonella phage SEN34]	187	187	100 %	2.00E-59	58.92 %	YP_009191616.1
		hypothetical protein 5.4 [Burkholderia phage Bups phi1]	133	133	94%	1.00E-38	44.94 %	ABY40559.1
33	127_aa|+|22652|23035	hypothetical protein 7AX3_36 [uncultured Caudovirales phage]	223	223	100 %	4.00E-75	84.25 %	ASN68774.1
		hypothetical protein [Klebsiella phage ST405-OXA48phi1.2]	192	192	97%	4.00E-63	75.00 %	QBP08204.1
		head-tail adaptor [Klebsiella phage ST16-OXA48phi5.2]	192	192	97%	1.00E-62	75.00 %	QBP28323.1
34	180_aa|+|23028|23570	hypothetical protein 7AX3_37 [uncultured Caudovirales phage]	272	272	100 %	7.00E-93	70.72 %	ASN68775.1

		hypothetical protein [Phage NG54]	230	230	100 %	3.00E -76	58.56 %	AZF92918.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	216	216	100 %	8.00E -71	54.44 %	QBP07885.1
35	381_aa|+|23574|24719	hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	575	575	100 %	0	72.18 %	QBP07886.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	572	572	100 %	0	72.44 %	QBP28325.1
		XkdK-like protein [Klebsiella phage ST405-OXA48phi1.2]	570	570	100 %	0	72.18 %	QBP08206.1
36	147_aa|+|24732|25175	hypothetical protein 7AX3_39 [uncultured Caudovirales phage]	263	263	97%	2.00E -90	85.31 %	ASN68777.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	261	261	100 %	2.00E -89	84.35 %	QBP28326.1
		hypothetical protein SEN34_14 [Salmonella phage SEN34]	256	256	98%	9.00E -88	82.76 %	YP_009191620.1
37	143_aa|+|25179|25610	hypothetical protein 7AX3_40 [uncultured Caudovirales phage]	204	204	92%	3.00E -67	72.73 %	ASN68779.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	167	167	97%	2.00E -52	58.99 %	QBP28327.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	167	167	95%	2.00E -52	59.56 %	QBP07888.1
38	49_aa|+|25646|25795	hypothetical protein 7AX3_41 [uncultured Caudovirales phage]	86.7	86.7	100 %	3.00E -23	83.67 %	ASN68780.1
		NTP pyrophosphohydrolase [Klebsiella phage ST11-VIM1phi8.3]	80.9	80.9	100 %	4.00E -21	75.51 %	QBP07889.1
		NTP pyrophosphohydrolase [Klebsiella phage ST16-OXA48phi5.2]	74.3	74.3	100 %	2.00E -18	73.47 %	QBP28328.1
39	667_aa|+|25792|27795	hypothetical protein 7AX3_42 [uncultured Caudovirales phage]	835	835	99%	0	61.89 %	ASN68781.1
		lytic transglycosylase domain-containing protein [Klebsiella	818	818	99%	0	61.73 %	QBP07890.1

		phage ST11-VIM1phi8.3]						
		tail tape measure protein [Salmonella phage SEN34]	780	780	98%	0	59.44 %	YP_009191622.1
40	205_aa|+|27795|28412	hypothetical protein [Klebsiella phage ST405-OXA48phi1.2]	259	259	98%	8.00E-87	61.46 %	QBP08210.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	257	257	98%	3.00E-86	60.98 %	QBP07891.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	257	257	98%	3.00E-86	60.98 %	QBP28330.1
41	100_aa|+|28409|28711	hypothetical protein SEN34_18 [Salmonella phage SEN34]	106	106	100 %	8.00E-30	46.00 %	YP_009191624.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	105	105	100 %	2.00E-29	48.00 %	QBP28331.1
		hypothetical protein [Klebsiella phage ST405-OXA48phi1.2]	105	105	100 %	2.00E-29	48.00 %	QBP08211.1
42	345_aa|+|28714|29751	hypothetical protein [Klebsiella phage ST405-OXA48phi1.2]	548	548	100 %	0	74.49 %	QBP08212.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	532	532	96%	0	74.47 %	QBP07893.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	529	529	98%	0	73.45 %	QBP28332.1
43	114_aa|+|29748|30092	hypothetical protein [Klebsiella phage ST405-OXA48phi1.2]	172	172	99%	3.00E-55	69.03 %	QBP08213.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	137	137	98%	1.00E-41	53.10 %	QBP07894.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	130	130	80%	6.00E-39	58.70 %	QBP28333.1
44	237_aa|+|30199|30912	translation initiation factor IF-2 [Klebsiella phage ST16-OXA48phi5.2]	370	370	99%	1.00E-129	75.32 %	QBP28335.1
		baseplate assembly protein [Salmonella phage SEN34]	360	360	96%	1.00E-125	77.29 %	YP_009191628.1
		translation initiation factor IF-2 [Phage NG54]	283	283	92%	4.00E-95	65.00 %	AZF92909.1

45	117_aa|+|30912|31265	hypothetical protein 7F9_2 [uncultured Caudovirales phage]	206	206	100 %	9.00E-69	83.76 %	ASN67657.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	202	202	100 %	5.00E-67	80.34 %	QBP07898.1
		hypothetical protein [Klebsiella phage ST16-OXA48phi5.2]	201	201	100 %	1.00E-66	81.20 %	QBP28336.1
46	399_aa|+|31265|32464	baseplate J superfamily protein [Klebsiella phage ST16-OXA48phi5.2]	605	605	98%	0	75.19 %	QBP28337.1
		hypothetical protein [Klebsiella phage ST405-OXA48phi1.2]	599	599	98%	0	74.43 %	QBP08217.1
		hypothetical protein SEN34_25 [Salmonella phage SEN34]	598	598	100 %	0	77.94 %	YP_009191631.1
47	226_aa|+|32461|33141	hypothetical protein SEN34_26 [Salmonella phage SEN34]	380	380	100 %	6.00E-134	79.20 %	YP_009191632.1
		hypothetical protein 7F9_4 [uncultured Caudovirales phage]	350	350	100 %	5.00E-122	74.78 %	ASN67659.1
		hypothetical protein [Klebsiella phage ST11-VIM1phi8.3]	281	281	98%	3.00E-94	56.20 %	QBP07900.1
48	460_aa|+|33141|34523	tail fiber protein [Salmonella phage SEN34]	211	211	33%	6.00E-62	67.74 %	YP_009191633.1
		hypothetical protein 7AX3_51 [uncultured Caudovirales phage]	113	113	18%	2.00E-26	65.12 %	ASN68791.1
		hypothetical protein 7F9_5 [uncultured Caudovirales phage]	113	113	18%	2.00E-26	65.12 %	ASN67660.1
49	130_aa|+|34523|34915	tail fiber assembly protein [Escherichia phage vB_EcoM_Goslar]	83.2	83.2	97%	1.00E-19	36.03 %	QBO63882.1
		tail fiber assembly protein [Enterobacter phage phiT5282H]	84.7	84.7	84%	2.00E-19	41.46 %	AYD79767.1
		tail fiber assembly protein [Erwinia phage EtG]	82.8	82.8	65%	8.00E-19	49.41 %	ASD51166.1
50	232_aa|-|35231|35929	unnamed protein product [Pseudomonas phage OBP]	31.2	31.2	9%	8.5	52.38 %	YP_004958074.1

51	307_aa|-|36528|37451	bactoprenol glucosyltransferase [Shigella phage SfIV]	555	555	99%	0	87.83 %	YP_008766890.1
		bactoprenol glucosyltransferase [Enterobacteria phage SfV]	553	553	100 %	0	86.32 %	NP_599056.1
		bactoprenol glucosyl transferase [Cronobacter phage ENT47670]	553	553	99%	0	87.17 %	YP_007237578.1
52	120_aa|-|37448|37810	RecName: Full=Bactoprenol-linked glucose translocase [Shigella phage SfX]	181	181	100 %	1.00E-58	68.33 %	Q9T1D7.1
		bactoprenol-linked glucose translocase [Salmonella phage SPN9CC]	173	173	100 %	2.00E-55	65.83 %	YP_006383842.1
		O-antigen conversion protein [Salmonella virus P22]	173	173	100 %	2.00E-55	68.33 %	NP_059583.1
53	62_aa|-|37875|38063	No significance						
54	82_aa|+|38568|38816	ubiquitin carboxyl-terminal hydrolase [Edafosvirus sp.]	30	30	81%	7.7	30.00 %	AYV78441.1

SP Table 2: EAP2 Annotation

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CURRICULUM VITAE

TIFFANY PAN

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EDUCATION

Master of Science in **Molecular Microbiology and Immunology**
Certificate in **Epidemiology for Public Health Professionals**
Bloomberg School of Public Health, Johns Hopkins University • June 2019

Bachelor of Science in **Ecology, Evolution, and Behavior**
Minor in **Interdisciplinary Design** and **Animal Behavior**
College of Biological Sciences, University of Minnesota- Twin Cities • May 2016

RESEARCH EXPERIENCE

Lab of Dr. George Dimopoulos
Master's Research *October 2017 – June 2019 • Baltimore, MD*

- Planned, developed, and carried out protocols, methods, and studies to investigate the effect of bacteriophages on the mosquito midgut microbiome and mosquito development as relating to vector borne disease and vector competence
- Practiced a variety of microbiology, molecular biology, and bacteriology techniques including PCR and isolating bacterial phages
- Used programs such as Chromas Pro and SnapGene to analyze the genomes of isolated bacteria and bacteriophages
- Experience with rearing, handling, and dissecting lab mosquitos
- Used standard statistical principles and techniques such as analysis of variance and regression and correlation analysis to analyze results using R and Excel
- Presented at the 2018 Entomological Society of America Meeting and the 2019 American Association for the Advancement of Science Meeting

The Global Public Health Observatory under Dr. Carlos Castillo-Salgado
Research Assistant *September 2018 – Present • Baltimore, MD*

- Conducted literature reviews to evaluate current knowledge and research in infectious disease epidemiology and disease surveillance using social media sites and surveillance systems and techniques used to monitor the opioid crisis
- Interpreted scientific findings and reviewed findings for technical accuracy
- Assisted with health assessments of undocumented grade school students in Baltimore

Lab of Dr. Raymond Koehler
Research Technologist *June 2017 – October 2017 • Baltimore, MD*

- Assisted with post-doctorate projects in pediatric traumatic brain injury and the effects of tuberculosis in the brain
- Preformed neurobehavioral tests of sensorimotor and cognitive function
- Processed brain tissue using Cryostat protocols for histology, immunohistochemistry, and immunoblotting
- Worked with rat and rabbit models

Lab of Dr. Fumiaki Katagiri
Lab Assistant *August 2015 – June 2016 • Minneapolis, MN*

- Mapped the Quantitative Trait Locis (QTL's) regarding plant immunity in *Arabidopsis thaliana* using R
- Lab management responsibilities included planting and caring for lab-reared plants, counting bacterial colonies to determine plant resistance, supervising undergraduate students in the lab, and training in new students as they joined the lab
- Coordinated the actions of a multi-disciplinary team to develop a new procedure to find QTL's for other traits

- Developed highly effective written communication skills in order to communicate between students and duties required in the lab
- Presented at the 2015 American Society of Plant Biologists (ASPB) Annual Meeting

RESEARCH PROJECTS

Population change research under Dr. Emilie Snell-Rood *2015 • Minneapolis, MN*

- Researched the effects of population density change on the selection of the butterfly reproductive structure
- Presented at the 2016 Animal Behavior Society (ABS) Annual Meeting and have been accepted to the journal of Behavioral Ecology

Parasitology research under Dr.Brian Wisenden and Dr. Sehoya Cotner *2014 • Itasca State Park, MN*

- Developed and carried out experiments investigating the relationship between fish behavior and parasite loads
- Developed protocols, worked, and troubleshot independently
- Presented at the 2015 Society of Experimental Biology (SEB) Annual Meeting and published in *Zebrafish* in 2016

PUBLICATIONS

Espeset, A.; Kobiela, M.; Sikkink, K.; **Pan, T.**; Roy, C.; Snell-Rood, Emilie. (2019). Anthropogenic increases in nutrients alter sexual selection dynamics: a case study in butterflies. Behavioral Ecology, *in press*

Pan, T., Gladen, K., Duncan, E. C., Cotner, S., Cotner, J. B., McEwen, D. C., & Wisenden, B. D. (2016). Bold, Sedentary Fathead Minnows Have More Parasites. Zebrafish, 13(4), 248-255. DOI: 10.1089/zeb.2015.1185

CONFERENCE ABSTRACTS

Evaluation of Bacteriophage Influence on Mosquito Larval Development. Entomological Society of America Annual Joint Meeting. Vancouver, British Columbia, Canada. 2018.

Effect of Population Density on Sexual Selection in the Cabbage White Butterfly. Animal Behavior Society (ABS) Annual Meeting. Columbia, Missouri. 2016.

Mapping Genes of the Arabidopsis thaliana that Affect Immunity Variation. American Society of Plant Biologists (ASPB) Annual Meeting. Minneapolis, Minnesota. 2015

Bold, Sedentary Fathead Minnows have More Parasites. The Society of Experimental Biology (SEB) Annual Meeting. Prague, Czech Republic. 2015.

PROFESSIONAL EXPERIENCE

Maryland New Directions

Research Intern *January 2018 – May 2018 • Baltimore, MD*

- Researched current and potential transportation options for those living in Baltimore city through a combination of community outreach and communication, on -the-ground fieldwork of the transportation system, and online research.
- Sought partnerships with other community organizations to collaborate to come up with potential solutions to obstacles in transportation access
- Presented a report on the current status, assessments, findings, cost and schedule changes, milestones, and project status of public transportation options in Baltimore and possible improvements

Chesapeake and Ohio Canal National Historic Park

Biological Science Intern *August 2016 – March 2017 • Hagerstown, MD*

- Conducted research to study and monitor the insect populations in the shale barrens, an important Appalachian habitat, and studied their correlations with temperature, plant populations, and how different insect genera influence each other

- Assisted with the development of biological study proposals, survey designs, and scopes-of work and conduction of biological field investigations involving wildlife species and habitats
- Participated in various animal and land surveys to create three invasive vegetation and restoration plans
- Assisted with public outreach activities preparing materials and informally responding to requests for information.
- Assisted with organizing and implementing volunteer events within the park. Taught volunteers basics in invasive plant identification and proper removal

Teaching Assistant
The Evolution of Sex (BIOL 1003) *January 2015 – May 2016 • Minneapolis, MN*
Ecology (BIOL 3807) *May – June 2015 • Itasca State Park, MN*
Animal Behavior (BIOL 3811) *May – June 2015 • Itasca State Park, MN*

- Developed skills to effectively communicate lessons and ideas to a broad range of students
- Responsibilities include instructing students through labs, grading assignments, and tutoring students through confusing material from the lab
- Taught relevant skills necessary for field research and biology at Itasca State Park

MicroPort Medical- Shanghai Subsidiary
Resource and Development Intern *June 2014 – August 2014 • Shanghai, China*

- Read through papers, procedures, and previous research for current projects. Compared current procedures to the requirements needed and edited three translated protocols
- Navigated Chinese language and cultural barriers
- Worked in a large team to push two protocols into final review

LEADERSHIP AND VOLUNTEER EXPERIENCE

Johns Hopkins School of Public Health Student Assembly
Treasurer *May 2018 – Present • Baltimore, MD*

- Responsible for budget planning and financial recording for the School of Public Health's Student Assembly
- Head of the Finance Committee and responsible for leading meetings to evaluate student group budget applications
- Negotiated a higher budget for Student Assembly from the Hopkins' Student Affairs office

Minnesota Public Interest Research Group (MPRIG)
Co-Chairleader *October 2012 – May 2015 • Minneapolis, MN*
(https://gopherlink.umn.edu/organization/95)

- Participated in democratic and governmental processes to push bills through the Minnesota State government
- Developed strong communications skills by engaging with students on campus and larger neighborhood community
- Responsibilities include leading weekly meetings, organizing students to take action on proposed issues affecting the community, and working to attain funding and grants for the group

ADDITIONAL SKILLS

Adobe Photoshop, Dreamweaver, InDesign, and Illustrator • R and STATA • ArcGIS and QGIS • HTML and CSS coding • Microsoft Office Suite and Macintosh equivalents • Google Office Suite